VOLUME 13 NU

FEBRUARY, 1923

Phytopathology

Organ of the

American Phytopathological Society

CONTENTS

of Rubus ..... B. O. Dodge 49

THOMAS S. HEADDEN AND ... Gardner 54

... Pepper and rust in produce resistance ... 65

... Reynolds ... 67

... ALLEN ... REGULA ...

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

... LANCASTER, PA.

Entered as ... matter at the post office at Lancaster, Pa.

... per year. Foreign postage and dependencies, Mexico and Cuba, ... per year. Canada ... per year.

DIFCO

DEHYDRATED CULTURE MEDIA

for the

PLANT PATHOLOGIST and MYCOLOGIST

BACTO-CORN MEAL AGAR DEHYDRATED

The water soluble ingredients of CORN MEAL modified with BACTO-AGAR ... pound makes more than 20 liters of medium.

BACTO-PRUNE AGAR DEHYDRATED

A clarified infusion of the best grade of PRUNES solidified with BACTO-AGAR ... pound makes more than 20 liters of medium.

PACKED IN QUARTER AND ONE POUND BOTTLES, IN POWDER FORM.

Specify "DIFCO"

The Trade Name of the Pioneer in the Research and Development of Bacto-Peptone and Dehydrated Culture Media.

DIGESTIVE FERMENTS COMPANY

DETROIT, MICHIGAN, U. S. A.

SEVENTH EDITION

Jordan's Bacteriology

Staining methods, etc.	Bacillus leprae.
Classification.	Glanders.
Bacteria and disease.	Other bacilli.
Immunity.	Spirilla.
Staphylococci.	Spirochetes.
Streptococci.	Trichomycetes.
Pneumococci.	Blastomycetes.
Meningococci.	Hyphomycetes.
Gonococci.	Pro ozoa.
Anthrax bacillus.	Filterable viruses.
Diphtheria bacillus.	Bacteriology of milk.
Colon-typhoid bacilli.	Nitrogen cycle.
Hemorrhagic septicemia.	Bacteria in arts and industries.
Hemophilic bacteria.	Bacteria of soil, air, and water.
Anaërobes.	Bacterial plant diseases.
Tubercle bacillus.	Infectious of unknown causation.

By Edwin O. Jordan, Ph. D., University of Chicago. Octavo of 744 pages, illustrated. Cloth, $5.00 net.

W. B. SAUNDERS COMPANY

PHILADELPHIA and LONDON

The Surest and P... For Dusting and B...

For dusting and fruit blossoming, the "ANCHOR" BRAND SUBLIMED VELVET FLOWERS and the "EAGLE" BRAND SUBLIMED FLOWERS OF SULPHUR are superior and will give the best and quickest results. They are SNOW-FLAKE-LIKE particles that cling to every surface and every part of the plant. They are the highest grades of sublimed sulphur produced. Do not adulterate these sulphurs with Lime, Kaolin, or any other inert material. Coating the sulphur with this inert material PREVENTS the FUMIGATION caused by the sun's rays.

For increasing crop yields, combating of potato scab, sugar beet scab, sweet potato scurf, onion thrip, etc., and for repelling cut worms, wire-worms, etc., which cause root knot and other root diseases, use "TOBACCO" BRAND SPECIALLY PREPARED AGRICULTURAL COMPOUND.

Send for circulars Nos. 5, 10 and 15 regarding dusting and agricultural compounds.

Stauffer Chemical Company of Texas

711 Scanlan Building

Houston Texas

Purchase from our advertisers. Mention Phytopathology when ordering.

American Phytopathological Society

President, G. R. Lyman, West Virginia College of Agriculture, Morgantown, W. Va.
Vice-President, F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.
Secretary-Treasurer, R. J. Haskell, U. S. Dept. of Agriculture, Washington, D. C.

ADDITIONAL MEMBERS OF THE COUNCIL

I. E. Melhus, College of Agriculture, Ames, Iowa.
M. F. Barrus, College of Agriculture, Ithaca, N. Y.
E. C. Stakmen (ex officio), University of Minnesota, St. Paul, Minn.
Perley Spaulding (ex officio), U. S. Dept. of Agriculture, Washington, D. C.
W. P. Fraser, University of Saskatchewan, Saskatoon, Saskatchewan, representing the Canadian Division.
F. D. Heald, State College of Washington, Pullman, Washington, representing the Pacific Coast Division.
L. R. Hesler, University of Tennessee, Knoxville, Tenn., representing the Southern Division.

Phytopathology

Official Organ of the Society

EDITORS

Perley Spaulding, Editor-in-Chief, U. S. Department of Agriculture, Washington, D C., 1921–23.
L. L. Harter, Assistant Editor-in-Chief, U. S. Department of Agriculture, Washington, D. C., 1922–23.
N. J. Giddings, West Virginia College of Agriculture, Morgantown, W. Va., 1923.

ASSOCIATE EDITORS

G. R. Bisby, University of Manitoba, Winnipeg, Canada, 1921–23.
J. Franklin Collins, Brown University, Providence, R. I., 1921–23.
L. R. Hesler, University of Tennessee, Knoxville, Tenn., 1921–23.
A. G. Johnson, U. S. Department of Agriculture, Washington, D. C., 1921–23.
Howard S. Fawcett, Citrus Experiment Station, Riverside, Calif., 1922–24.
W. P. Fraser, University of Saskatchewan, Saskatoon, Saskatchewan, 1922–24.
F. A. Wolf, North Carolina Agricultural College, West Raleigh, N. Car., 1922–24.
Leo E. Melchers, Kansas Agricultural College, Manhattan, Kansas, 1922–24.
J. G. Leach, College of Agriculture, University Farm, St. Paul, Minn., 1923–25.
W. D. Valleau, University of Kentucky, Lexington, Ky., 1923–25.
R. D. Rands, U. S. Department of Agriculture, Washington, D. C., 1923–25.
W. A. McCubbin, Bureau of Plant Industry, Harrisburg, Pa., 1923–25.

BUSINESS MANAGEMENT

R. J. Haskell, Business Mgr., U. S. Dept. of Agriculture, Washington, D. C.
Roy G. Pierce, Advertising Mgr., U. S. Dept. of Agriculture, Washington, D. C.

NOTICES

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity and conciseness are essential. In form and style manuscript should conform to the best usage in this journal. It should be typewritten on one side of the paper and sent unfolded.

Illustrations necessarily must be limited in number, and photographs, to reproduce satisfactorily, must be of the best quality. Line drawings reproduce best as text figures. Authors desiring unusual numbers of illustrations or special types of reproduction will be asked to bear part of the expense.

Subscription price. $5.00 per year United States and dependencies, Mexico and Cuba; Canada $5.25; other countries $5.50; current single numbers 50 cents. The journal is issued monthly beginning with Vol. VIII, January, 1918.

Requests to supply lost copies of the journal must be made within 60 days from date of issue. Date of issue January 1 and monthly thereafter.

Separates. No gratis copies are supplied. A printed schedule of prices is submitted with the proof and authors may secure separates by placing an order when proof is returned.

Back Volumes may be obtained unbound at $6.00 per volume, postage paid.

Separate copies will not be sold except in cases where the volumes are already broken. The price of such copies is $1.00 each for Volumes I to VII inclusive, and 50 cents each for subsequent years. Prices net postpaid.

NON-CORROSIVE

MICRO SLIDES AND COVERS

THE TEST

IN OUR STOCK FOR IMMEDIATE SHIPMENT

OF SERVICE

7020

7022

7030

7020. Micro Cover Glasses, Squares, Non-Corrosive, Red Label, of hard crown glass of slightly greenish tint when viewed edgewise, guaranteed against corrosion in any climate. These are the only Cover glasses we are willing to guarantee against corrosion under all conditions and should not be confused with lower priced glasses made of soft white glass. From the same source and of exactly the same quality as sold by us for the past twenty years. Neatly packed in ½-ounce round wooden boxes with conspicuous red label. Please specify size and thickness in ordering.

Size, mm	12	15	18	22	25
No. 1 (from 0.13 to 0.17 mm. thick), **per ounce**	2.00	1.80	1.80	1.80	1.80
No. 2 (from 0.17 to 0.25 mm. thick), **per ounce**	1.75	1.40	1.40	1.40	1.40

10% discount in cartons containing 25 ounces = 50 boxes.
20% discount in cases containing 100 ounces = 4 cartons = 200 boxes.

7022. Micro Cover Glasses, Circles, Non-Corrosive, Red Label, same quality as No. 7020. Packed in ½-ounce round wooden boxes.

Diameter, mm.	12	15	18	22	25
No. 1, per ounce	2.75	2.40	2.40	2.40	2.40
No. 2, per ounce	2.25	1.85	1.85	1.85	1.85

10% discount in cartons containing 25 ounces = 50 boxes.
20% discount in cases containing 100 ounces = 4 cartons = 200 boxes.

7030. Micro Slides, Non-Corrosive, Red Label, Special Selection, of hard glass, slightly greenish tint when viewed edgewise. Guaranteed against corrosion in any climate. With well ground edges and of fair uniformity in thickness, mostly about 1 mm. We recommend this slide as the best micro slide now available. Size 3 x 1 inch; packed in ½ gross cartons. **Per gross** **1.45**
10% discount in case containing 25 gross = 50 cartons.

7030a. Micro Slides, Non-Corrosive, Red Label, Ordinary Selection, differing from No. 7030 only in uniformity of thickness, edge grinding and freedom from scratches. These slides are equal to or superior in quality to the ordinary trade articles. **Per gross** ..**1.20**
10% discount in cases containing 25 gross = 50 cartons.

7033. Micro Slides, Non-Corrosive, Red Label, exactly same as No. 7030, but size 3 by 2 inches; packed in ½ gross cartons. **Per gross** **2.40**
10% discount in case containing 12½ gross = 25 cartons.

NOTE—We also carry in stock Non-Corrosive Cover Glasses in thirteen rectangular sizes, in both No. 1 and No. 2 thickness.

Prices subject to change without notice

ARTHUR H. THOMAS COMPANY

WHOLESALE, RETAIL AND EXPORT MERCHANTS

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA, U. S. A.

CABLE ADDRESS, "BALANCE," PHILADELPHIA

PHYTOPATHOLOGY

VOLUME XIII NUMBER 2

FEBRUARY, 1923

THE DISTRIBUTION OF THE ORANGE-RUSTS OF RUBUS

B. O. Dodge

In connection with a line of work primarily devoted to infection experiments with the orange-rusts of Rubus, some observations were made on the occurrence of the long-cycled form, Gymnoconia, at various localities, in regions considerably south of its hitherto known range. The distribution of the short-cycle rusts has been the subject of much study, some holding that they are more common in the northern regions where the shortness of the season tends to eliminate the accessory spore forms. It is not unlikely that conditions limiting the geographical range of the micro- or lepto-puccinias are entirely different from the factors that determine the distribution of the Endophyllum-like short cycled rusts. As pointed out by Arthur (1) the orange-rusts furnish us with a subject, a thorough investigation of which may serve to clear up some of these highly important questions. It is necessary, therefore, to have at hand as far as possible, data regarding the distribution of both rusts, and it is with this in mind that the following notes are submitted. The writer has had the enthusiastic support of Drs. N. E. Stevens and C. L. Shear, who have contributed materially toward furthering this work.

Arthur (1) listed the collections of the orange-rusts in his herbarium either as *Kunkelia nitens* (Schw.) Arth., or as *Gymnoconia interstitialis* (Schl.) Lagh., "more or less arbitrarily," the known distribution of the telial stage being the prime factor in determining whether a given collection of the aecial stage should be listed as the one or the other. The Gymnoconia is not shown to occur west of the Mississippi, except in Alaska, otherwise it was supposed to be confined to the region east of the Mississippi and in general north of Ohio, or in the northeastern United States and southeastern Canada. Arthur thinks that there can be no doubt that the northern-most area is occupied by the long-cycled rust and the southern-most area by the short-cycled form. Differences

in the morphological characters of the aeciospores (7) which frequently enable one to predict before germination tests are made which form is at hand, had not been pointed out at that time and germination studies had been made in only a few localities.

Atkinson (3) claims, in effect, that low temperatures inhibit the production of promycelia, so that the nature of the orange-rust would be long-cycled or short-cycled, depending on the temperature at the time of the germination of the aeciospores, a statement easily disproved by a simple experiment.

Kunkel (7) found that the rust on the black raspberry in the vicinity of Washington, D. C., is always long-cycled, and states that the blackberries and dewberries in the same region are attacked only by the short-cycled form. This, if true, would suggest that specific differences in the hosts might also be an important factor in limiting the range of each rust. On the other hand, the long-cycled form was found on blackberry much farther south, at Mountain Lake, Virginia, which is at an altitude of about 3,000 feet. This could be cited as evidence that higher altitudes as well as higher latitudes are more favored by the Gymnoconia. He says that the aeciospores of the long-cycled form occasionally produce promycelia and do so more frequently when germinated at the low temperatures, 5–10° C., such as one finds in the ordinary ice box. This author did not study the nuclear phenomena in connection with the germinating spores which produced these structures and does not claim that he observed a nuclear fusion, but he assumes that such is the case since he finds what appear to be normal promycelia. Sappin-Trouffy (9) pointed out in a paper overlooked by Kunkel that the aeciospores as well as the uredospores of a number of rusts may germinate rather abnormally. Branched germ tubes with cross walls and supernumerary nuclei are not uncommon in cultures of germinating rust spores. The writer (6) also had described and figured in detail a curious type of germination of aeciospores. As the aeciospore of *Gymnosporangium transformans* germinates the two nuclei may come to lie in a pouch-like enlargement of the germ tube where they divide. The end of the tube grows out and branches more or less irregularly, often forming three or four short divisions into each of which a single nucleus may migrate. A definite cross wall cuts off the forward part of the tube which contains most of the granular cytoplasm from the portion next to the spore. As the culture ages large vacuoles separated by fine lines of cytoplasm help to give these germ tubes the appearance of promycelia. The writer has not studied the germination of the aeciospores of the Gymno-

conia very extensively and has not seen any true promycelia although branched germ tubes are not rare. The two orange-rusts are certainly so closely related that one must have been derived from the other, either by a previous fusion of the nuclei in the aeciospore germ tubes, as suggested by the double life history of the Gymnoconia observed by Kunkel, or by a delayed fusion followed by the development of a telial stage in a short-cycled form.

Clinton (4) states that both forms occur at New Haven, Connecticut, and Davis (5) now finds the short-cycled form in Wisconsin, where only the Gymnoconia was supposed to exist. Just why only Kunkelia has been found in Minnesota, while across the river in Wisconsin the Gymnoconia is so prevalent, is not clear.

While on a trip to North Carolina in 1921, the telial stage of Gymnoconia was found, October 10, on *Rubus argutus* at Ridge Crest, (altitude about 2600 ft.) in the vicinity of Asheville. One of the systemically infected plants was brought to Washington and planted. Aecia matured about May 1, and the spores on germination showed only long, negatively heliotropic germ tubes,—no promycelia.

From April 19–24, 1922, many collections of orange-rust were made at Winston-Salem, North Carolina, in the region where Schweinitz first found *Aecidium nitens*. The long-cycled rust was found on *Rubus occidentalis* back of the Moravian Seminary. All orange-rust found here at this time on blackberry and dewberry proved to be short-cycled.

At Salisbury, North Carolina, April 25, about twenty collections of aecia of a very dark reddish-orange color on blackberry were made. The color of the rust seems to be a very deceptive character, as all found on blackberry here also proved to be short-cycled.

April 25 and 26 some fifty collections of the rusts on blackberries were made in North Carolina between Ridge Crest and Old Fort which is at an altitude of about sixteen hundred feet. Two of the collections on wild blackberry proved to be long-cycled, one obtained at the crest, the other at the base. Four other collections were too young to determine. Black raspberries in the vicinity along the railroad near Old Fort were well infected with the Gymnoconia form. On this host aecia were just beginning to mature, while the short-cycled form on blackberries was well out and powdery wherever found. Thinking that perhaps in some localities the Gymnoconia might mature a little later, several blackberries having only the pycnial stage at Ridge Crest were marked and on May 17, three weeks later, Rev. J. L. Ray sent rusted leaves from these plants. With one exception they bore the

long-cycled form of the rust. It has also been noted that where both rusts were growing on the same species of dewberry (originally obtained in an infected condition from North Carver, Massachusetts) at Arlington, Virginia, the Kunkelia form develops at least a week earlier than the Gymnoconia. How far this will prove to be true another year, or in general, of course, must be determined by further observation. It is not improbable, however, that collections made about May 15, at Salem and Bethania would also result in the discovery of the Gymnoconia on blackberry in the region covered by Schweinitz.

At Washington further search was made for the Gymnoconia on the blackberry and dewberry. On May 24, along the bottom lands of the Patuxent River at Hill's Bridge near Leon, Maryland, it was noticed that the rust on some of the blackberries was a rather dark reddish orange. The two specimens obtained at the time proved to be long-cycled. Three days later sixty collections were made along this roadside and in an abandoned field one mile east. It was easy to determine by the size, shape and color of the spores which form was under observation, a mistake being made in only one case. About half of the infected blackberries along a filling of sand and gravel bore the long cycled rust. At Pindell cross road the short-cycled rust predominated at one end of the field, fully 50 per cent of the plants being infected. A few rods farther on many plants were infected with the Gymnoconia. The black raspberries in the immediate vicinity seemed to be entirely free from the rust. Several infected blackberry plants were transferred to the greenhouse where telia appeared in July on those that survived. An abundance of teliospore material was collected at the Patuxent River locality, July 22 and August 17.

About a dozen infected wild blackberry plants had been obtained near Forestville, Maryland, a few miles from Washington, on April 11. It took some time for them to recover after being potted, but by May 31 most of them had mature aecia on the shoots that had grown out since transplanting some light orange in color, others a darker reddish orange. Germination tests proved that one plant, No. 291, had the long cycled rust; two others, Nos. 292 and 296, had a rust whose spores were large spherical, of uniform size, and a dark reddish-orange color. These spores produced at once negatively heliotropic germ tubes, which after growing out vigorously for some distance became very regularly perfect promycelia bearing sporidia. Several other plants from this place developed the more typical Kunkelia. Telia were found at Forestville, Maryland, July 22, where these plants had originally been obtained.

Several collections of orange-rust were made at Bethlehem, Pennsylvania, June 10 to 12. A number of plants of *Rubus occidentalis* bore a light orange colored rust which proved to be long-cycled. A number of wild blackberries also bore the Gymnoconia. On the hill above Lehigh University, two blackberries bearing the long-cycled rust grew near ten others infected with the short cycled form. These could be distinguished by the color of the rust, although germination tests of the spores are always necessary to prove each case.

Five specimens of rust were collected on blackberry by Dr. Shear at Dover, Ohio, June 17, 1922. The two on the cultivated variety, Eldorado, proved to be the Gymnoconia, while one each on a wild blackberry, *Rubus allegheniensis*, and a cultivated variety, Mercereau (?), were short cycled. April 25, a specimen of Kunkelia was received from Oklee, Minnesota, on cultivated blackberry. Oklee is in 48° north latitude where the winters are notably very severe.

On May 12, 1921, Dr. Stevens sent in several infected plants from Woodstock, New York. In each case tests proved that the rust on blackberries and dewberries was short-cycled, but on the black raspberry the rust was long-cycled. The same region was visited on May 30, 1922. One collection on blackberry at 2,000 feet was long-cycled, while one made on wild dewberry at 1,000 feet was short-cycled. The rust on *Rubus occidentalis* at both of these altitudes was long-cycled. Random collections made at North Carver, Mass., May 27, 1921, showed that both forms grow here on blackberry side by side, five specimens being Kunkelia and five Gymnoconia. Several collections made July 8, the same year, from this locality proved to be long-cycled. The Gymnoconia has been found in Maine at Wells, North Livermore, Leeds Junction, Lewiston and Phillips on *Rubus canadensis* and other wild blackberries and dewberries. We have not found Kunkelia as yet in this state.

A great many germination tests have been made of aeciospores from *R. occidentalis* within a radius of twenty miles of Washington, D. C., without finding the Kunkelia on this host.

Experiments to be described elsewhere show that the following cultivated varieties of blackberries are susceptible to infection by the Kunkelia at Washington: Iceberg, Crystal White, Mercereau, Crandall, Kittatinny, Loganberry, Ward, Taylor and Eldorado (?). Telia of the Gymnoconia have been collected on leaves of the Ward, Loganberry, Eldorado, and Kittatinny, Mercereau, Taylor and the mountain blackberry, *R. canadensis*, in the same region.

The following table may be considered in the nature of a supplement to Kunkel's list of collections (7), the aeciospores of which were tested by germination. Where more than one collection was made at a given locality on a certain date the number tested is sometimes shown in parenthesis. While the color of the aecium is a character frequently helpful in predicting how the spores will germinate, it is not a constant feature and it is not mentioned in our table. For example, at Bethania, N. C., April 23, 1922, short cycled aecia on blackberry were a very dark reddish- or brownish-orange color; at Old Fort, N. C., April 25, the color of the same form varied from light lemon-yellow to deep xanthine orange, regardless of the state of maturity of the aecia.

TABLE 1

Place and time of collection, host and manner of germination of the aeciospores of various collections of orange rusts

Place of collection	Time of collection	Host	Manner of germination[1]
Wells, Me.	July 10, 1922	Wild blackberry	Germ tubes
Arlington, Va. (From Phillips, Me.)	May 1–10, 1922	Wild dewberry (14)	" "
Phillips, Me.	June 27, 1921	*R. canadensis*	" "
Wells, Maine	July 5, "	Wild dewberry (3)	" "
North Livermore, Me.	June 27, "	Wild blackberry (2)	" "
Portland Farms, Me.	July 5, "	" " (3)	" "
Leeds Jct., Maine	" 1, "	Wild dewberry (6)	" "
Lewiston, Me.	June 28, "	" " (7)	" "
Phillips, Me.	" 27, "	" "	" "
Phillips, Me.	July 8, "	" "	" "
N. Woodstock, N. H.	June 20, "	" " (4)	" "
N. Woodstock, N. H.	" 21, "	" " (3)	" "
Profile Lake, N. H.	" 24, "	Wild blackberry & wild dewberry (12)	" "
Bartlett, N. H.	July 11, 1922	Wild blackberry	" "
Franklin, N. H.	June 21, 1921	Wild dewberry	" "
Bartlett, N. H.	" 23, "	*R. canadensis* (7)	" "
Beamis, N. H.	" 21, "	*R. canadensis*	" "
Crawford Notch, N. H.	" 20, "	Wild blackberry (4)	" "
Woodstock, N. Y.	May 12, "	" " (8)	Promycelia
Woodstock, N. Y.	" 12, "	*R. occidentalis*	Germ tubes
Woodstock, N. Y. (1,000 ft. alt.)	" 31, 1922	Wild dewberry	Promycelia

[1] Miss Ruth Colvin made many of the germination tests here reported.

TABLE 1 (*Continued*).

Place of collection	Time of collection	Host	Manner of germination
Woodstock, N. Y. (1,000 ft. alt.)	May 31, 1922	*R. occidentalis*	Germ tubes
Woodstock, N. Y. (2,000 ft. alt.)	" 31, "	Wild blackberry	" "
Woodstock, N. Y. (2,000 ft. alt.)	" 30, "	*R. occidentalis*	" "
Marlboro, N. Y.	May 18, 1921	Wild Rubus spp. (5)	Promycelia
Pine Bush, N. Y.	May 28, 1922	Wild blackberry	Germ tubes
Bull Hill, N. Y.	May 28, "	Wild blackberry	" "
Bull Hill, N. Y.	May 28, "	Cultivated blackberry	Promycelia
Brooklyn, N. Y.	July 1, 1920	Wild blackberry	"
Reading, Mass.	" 3, "	Cultivated blackberry	"
North Carver, Mass.	May 27, 1921	Wild dewberry (3)	Germ tubes
North Carver, Mass.	June 6, 1921	*R. occidentalis* (3)	" "
North Carver, Mass.	" 6, "	Wild blackberry (2)	Promycelia
North Carver, Mass.	May 27, 1921	Wild dewberry (5)	"
Salisbury, Mass.	July 10, 1922	Wild blackberry (2)	Germ tubes
Pittsfield, Mass.	June 2. 1921	*R. occidentalis*	" "
Canaan, Conn.	May 20, "	Black raspberry (5)	" "
Hammonton, N. J.	June 5, 1922	Wild blackberry (2)	Promycelia
Cologne, N. J.	" 6, "	" " (3)	Promycelia
Bunola, Penn.	May 16, 1920	Cultivated blackberry	"
Bethlehem, Penn.	June 8, 1922	Wild blackberry (10)	"
Bethlehem, Penn.	" 8, "	" " (5)	Germ tubes
Bethlehem, Penn.	" 8, "	*R. occidentalis* (4)	" "
Bethlehem, Penn.	" 8, "	Wild dewberry (2)	Promycelia
Bell, Maryland (Holly Hill, Fla.)	May 15, "	Dewberry × blackberry	"
Bell, Md.	" " "	Ward blackberry	"
Bell, Md.	" " "	Mammoth dewberry (seedling)	"
Bell, Md.	" 15, "	*R. teterhizans*	Promycelia
Leon, Md.	" 24, "	Wild blackberry (2)	Germ tubes
Leon, Md.	" 24, "	*R. cuneifolius*	Promycelia
Leon, Md.	" 27, "	Wild blackberry (31)	Germ tubes
Leon, Md.	" 27, "	" " (29)	Promycelia
Forestville, Md.	" 27, "	" " (4)	Germ tubes
Forestville, Md.	" 27, "	" " (10)	Promycelia
Forestville, Md. (greenhouse plant)	" 31, "	" " (1)	Germ tubes
Forestville, Md. (greenhouse plants)	" 31, "	" " (7)	Promycelia

TABLE 1 (*Continued*).

Place of collection	Time of collection	Host	Manner of germination
Pasadena, Md.	May 24, 1922	*R. hispidus*	Promycelia
Pasadena, Md.	" 24, "	*R. cuneifolius*	"
Woodward, Md.	" 30, "	Wild blackberry (5)	"
Fort Myer, Va.	April , "	Cultivated blackberry	Germ tubes
Arlington, Va.	April , "	Loganberry	Promycelia
Bedford, Va. (3850 ft.)	July 2, "	Wild blackberry (2)	"
Vienna, Va.	May 30, 1921	Wild blackberry (2)	"
Vienna, Va.	" 10, "	*R. occidentalis* (4)	Germ tubes
Prospect Hill, Va.	" 22, "	*R. cuneifolius* (5)	Promycelia
Prospect Hill, Va.	" 22, "	Wild dewberry (3)	Promycelia
Old Fort, N. C. (1650 ft. alt.)	April 25, 1922	*R. canadensis* × *R. nigrobaccus*	Germ tubes
Ridgecrest, N. C.	" 26, "	*R. nigrobaccus* (?)	Germ tubes
Ridgecrest, N. C.	" 26, "	*R. nigrobaccus* (?) (30)	Promycelia
Ridgecrest, N. C.	May 17, "	*R. nigrobaccus* (?) (5)	Germ tubes
Ridgecrest, N. C.	" 17, "	*R. nigrobaccus* (?)	Promycelia
Salisbury, N. C.	April 24, 1922	Wild black raspberry (2)	Germ tubes
Salisbury, N. C.	" 24, "	Wild blackberry (20)	Promycelia
Old Fort, N. C.	" 25, "	Wild blackberry (many)	"
Winston-Salem, N. C.	" 20, "	Wild blackberry and dewberry (24)	"
Winston-Salem, N. C.	" 21, "	*R. occidentalis* (3)	Germ tubes
Bethania, N. C.	" 23, "	*R. argutus* (10)	Promycelia
Pilot Knob, N. C.	" 23, "	Wild blackberry (5)	"
Pilot Knob, N. C.	" 23, "	Wild dewberry(4)	"
Winston-Salem, N. C.	" 22, "	*R. Enslenii* (10	"
Winston-Salem, N. C.	" 22, "	*R. trivialis* (3)	"
Cameron, N. C.	" 17, "	Wild blackberry (11)	"
Charleston, S. C.	Mar. 21, "	Cultivated blackberry	"
Lotte, Fla.	Feb. 29, 1921	" "	"
Oklee, Minn.	June 23, 1922	Cultivated blackberry	"
South Haven, Mich.	" 22, "	*R. occidentalis*	Germ tubes
Rockford, Ill.	" 17, "	Cultivated blackberry	Promycelia
Dover, O.	" 19, "	Wild blackberry	"
Dover, O.	" 19, "	Eldorado blackberry (2)	Germ tubes
Dover, O.	" 19, "	R. *alleghaniensis*	Promycelia
Dover, O.	" 19, "	Mercereau? blackberry	"
St. Louis, Mo.	May 27, "	Cultivated blackberry	"
Fort Worth, Texas.	April. 2, 1921	Cultivated blackberry	"

Considerable uncertainty exists regarding the particular species of Rubus upon which Schweinitz first found what he called *Aecidium*

nitens. In his first published description of the rust (10) he cites *Rubus strigosus* as the host, and it is so cited in his first manuscript. In his later publication (11) he simply states that the rust is found on *Rubus*.

Arthur (1, p. 504) describes the new genus Kunkelia, citing Schweinitz's Salem collection as the type (Cf. Arth. & Bisby, 1918, p. 230). In the former paper Arthur makes the new combination as follows:—"*Kunkelia nitens* (Schwein.), comb. nov.—*Aecidium nitens* Schwein. Schrift. Nat. Gesell. Leipsig 1: 69–1822 (type on *Rubus* '*strigosus*' error for *R. Enslenii*, Salem, N. C.)." The discussion of their studies of the collections of the orange-rust in the Schweinitz Herbarium at the Philadelphia Academy by Arthur and Bisby (2) is not altogether clear. Schweinitz cites *R. strigosus* as the host in his *first* published account instead of the last, and the long-cycled rust has certainly been reported several times since 1822 on *R. strigosus*. It would be indeed a very small matter on just what particular species of Rubus Schweinitz first found *Aecidium nitens* if there were only one orange-rust on Rubus or even only one at Salem, N. C. But if both long- and short-cycled forms are today found at Salem, N. C., and much farther south, it is a question whether Schweinitz may not after all have first found the long-cycled form, not on the red raspberry *Rubus strigosus*, but on the black raspberry, *R. occidentalis*, which today can be found there infected with the Gymnoconia.

Arthur and Bisby (2) state that the orange rust in the Schweinitz Herbarium at Philadelphia is represented by five leaves in a mounted packet and by many leaves and leaflets in the original packet, which is labeled inside "Aecidium nitens in Rubo villoso Salem Bethl Neujork." These authors state that it was the custom of Schweinitz to preserve but the one original collection to represent each species. "It is quite evident from its appearance that the ample material of the present species was all gathered at one time and that it is all, or nearly all, from one plant as it is very uniform. A part of the material has been seen by Dr. P. A. Rydberg . . . and he states that the host cannot possibly be *R. strigosus*, but that it may be *R. procumbens* Muhl., or more likely, its southern representative *R. Enslenii* Walt., both of which usually passed under the name '*R. villosus*, a century ago."

Through the kidness of Mr. Eugene A. Rau of Bethlehem, Penn., Dr. Shear and the writer were able to examine an unpublished manuscript of Schweinitz entitled "Flora Salemitana sistens Plantas et Vegetabilia omnia huc usque in agro Salemitano Carolinae Septentrionalis observata Radio triginta millium ex Carolinae Septentrionalis Comitatibus Stokes, Surry, Guilford, Rockingham Et Rowan. Autore L. D. v. Schweinitz

1821 Aprili fine. In margine additas envenies Plantas quas ex Bethlehem Pennsylvania loco natali observatum." The following extract may serve to clear up some of the uncertainty as to Schweinitz's knowledge of species of *Rubus*.

Page 72	271	Rubus (Salem)
common 738		1. occidentalis our common raspberry.
common 739		2. villosus this appears to suit best our common blackberry.
common 740		3. trivialis the common dewberry.
rare 741		4. flagellaris rarer v. c. at Quaker.
not rare 742		5. obovalis at the double branches
hispidus Easton		
odoratus Easton, Bethl. Lehigh Mts.		
strigosus, Blue Mts.		
cunefolius, Hope (N. J.)		

This list indicates clearly that Schweinitz did not use the name *villosus* to include the dewberry as suggested by Rydberg in the above quotation. The specimen in the Schweinitz Herbarium at Philadelphia labeled *R. villosus* is a true blackberry and has been labeled *R. allegheniensis* by Blanchard and has been placed in the cover with *R. argutus*.

Dr. Shear, who is familiar with the work of Schweinitz and his methods, has also examined the collections of orange rust in the Schweinitz Herbarium at Philadelphia, and has furnished the following memorandum which throws more light on the question of the type of *Aecidium nitens*.

"Schweinitz probably used the name *Rubus strigosus* for the host upon which his first specimens of the orange rust were collected on account of a misunderstanding of the application of this name which had only a short time before (8, p. 296) been published by Michaux. A list of the botanical works in Schweinitz's library recently obtained by the writer through the kindness of Mr. Eugene A. Rau of Bethlehem, Pennsylvania, shows that this was one of the books that he used and the one from which he probably derived this name and the description. In the interval between 1817, when Schweinitz finished the manuscript of his Fungi of North Carolina, and 1821, when he prepared the above list of Rubi, he apparently discovered that he had applied the name *strigosus* erroneously to plants growing about Salem and therefore abandoned it as it does not occur in this list. Whether in this case, which is the only one we

can find where he used this name for specimens of Rubus from North Carolina, either as the host of a fungus or as a member of the flora, he applied it to *Rubus occidentalis* or to *R. villosus* of the older authors cannot perhaps be determined with certainty at present, as part at least of his original specimen of *Aecidium nitens* was sent to Schwaegrischen with his manuscript in 1817, and if extant its present location is unknown. In any case it is not at all probable that the original host was *R. strigosus*, as this species does not occur about Salem now and probably never did. It seems much more likely that Schweinitz applied the name erroneously to *R. occidentalis* rather than *R. villosus* or *R. procumbens* as Michaux's brief description states that the leaves of *strigosus* are white tomentose underneath and this would not apply to any of the blackberries or dewberries known to occur in Salem or vicinity.

An examination of such of Schweinitz's specimens of the rust as are available appears to throw some light on this question. While it does not seem possible for a Rubus expert to tell with much certainty the species of Rubus to which a leaf completely covered with aecia of the orange-rust belongs, yet it is perfectly practicable after considerable study and comparison of rusted leaves in the field to tell whether any particular specimen came from a raspberry as compared with a blackberry or dewberry. Rusted raspberry leaves are a different color and texture from rusted blackberry or dewberry leaves. The rusted raspberry leaves are thinner, more fragile and paler green color than the others.

Schweinitz's mycological collections when transferred to the Philadelphia Academy of Natural Science were preserved in small paper packets which were placed in large packets and tied in quarto board covers. Later, in 1854–1857 Michener removed, in some cases part and in some cases all, of the specimens from the original packets and glued them to squares of paper and mounted these in folio volumes. In the case of *Aecidium nitens* only a part of the material in Schweinitz's original packet was removed and mounted by Michener. The original packet bears the following data in Schweinitz's hand-writing:

"Aecidium nitens
in Rubo villoso
Salem Bethl. Neu York."

"There is evidence here, as in many other cases of Schweinitz's specimens which we have examined, that one of the leaves had originally been glued to the sheet in which the rusted leaves are now wrapped. As has already been pointed out (Endothia Parasitica and Related Species,

Bulletin 380, U. S. D. A., page 7, 1917) the evidence shows that Schweinitz at one time mounted his specimens of fungi by gluing them to sheets of paper and writing the labels on the sheets beneath the specimens. Later he changed his plan, removed the glued specimens from the sheets, cut up the paper on which a specimen had been mounted, folded it into a packet and placed the original specimen inside; and in many cases at least also added later other specimens from the same or different localities. On this particular packet, apparently later and with different ink, was written *Caeoma luminatum*, the name which he used in his later publication in 1832. In the original packet, as well as the mounted specimen, there are leaflets of two quite different species, one of them possessing the characters of rusted *R. occidentalis* leaves and the other clearly belonging to the blackberry-dewberry type. A leaf of the *occidentalis* type seems to be the one which was originally glued to the herbarium sheet, and if we were justified in assuming that this is a part of the type or the same gathering as that Schweinitz sent Schwaegrischen with the manuscript of his Syn. Fun. Car. Sup. there would be strong reason for believing that the type of the orange-rust was on *R. occidentalis* and, as at present we have found only the long-cycled rust on this host, it might be concluded that Schweinitz's name should be applied to the long-cycled rust. As, however, the evidence is not entirely conclusive, it is probably better to follow the precedent established by Arthur and apply Schweinitz's name to the short cycled rust."

In the case of rust collected on *Rubus* in April, 1922, at Salem, N. C., it was possible in several instances to obtain specimens of only partially rusted plants or of infected plants which were root sprouts from those free from rust and then in blossom. We are indebted to Dr. P. A. Rydberg for identifying the hosts as far as possible. It appears that today the Gymnoconia occurs on *R. occidentalis* at Salem, and that the Kunkelia is to be found there on *R. argutus*, *R. floridus*, *R. Boyntoni*, *R. trivialis* and *R. Enslenii*. We have no specimens of rust on Salem plants identified by Dr. Rydberg as *R. frondosus*, *R. betulifolius*, and *R. Bailey-anus*. The rust is more common on blackberries than on dewberries. If the rust on the black raspberry is always long-cycled as Kunkel found it to be in the vicinity of Washington, D. C., the distribution of the Gymnoconia probably coincides with the range of the host, *R. occidentalis*.

There is still much to be desired in the way of knowledge regarding the distribution of these rusts. Careful germination tests must be made in every case as there is no known constant morphological character by which the rusts can be distinguished. It would be especially instructive

to determine just how far north in the New England States the Kunkelia grows. Further investigation of the rust on the cultivated black raspberries west of the Mississippi should be made, as the Gymnoconia is more likely to be found on this host.

As one travels from Florida through the coast states to Maine, he is struck by the change from one orange rust to the other, the short-cycled clearly predominating in the South and the long-cycled in the North. In strong contrast, however, in the states along the western bank of the Mississippi, from Texas to Canada, the short-cycled, according to Arthur, alone occurs.

Many specimens of orange rust have been gathered in the White Mountains of New England during the latter part of June and the first week in July. We were therefore much surprised to find on July 10, 1922, that the aecial stage had almost entirely disappeared at Wells, Maine, and at Bartlett, New Hampshire. Specimens obtained showed green leaves covered with maturing spermogonia interspersed with telia. There were no signs of aecial scars on these leaves but such scars could generally be found on some of the fallen leaves at the base of infected plants. Did telia follow spermogonia so quickly on infected leaves as to entirely prevent the formation of aecia? It is presumed that such telia resulted from infection by aeciospores, but should cell fusions sometimes occur and these result in the development of telia we can see how a short-cycled Puccinia might arise.

What are the facts regarding the Gymnoconia on *R. strigosus* or Kunkelia on *R. occidentalis*? In view of the number of species of Rubus known to be susceptible to attack by both rusts we should expect that *R. occidentalis* would harbor the Kunkelia as sometimes reported. If Kunkelia does occur on this host, it must be only occasionally, as every collection, so far tested by the writer, has turned out to be the Gymnoconia. These rusts attack plants with all degrees of severity, frequently the second year the plants are so completely transformed that it is almost impossible to tell one infected species of blackberry or dewberry from another. This being the case, the specific names assigned to the hosts in no small percentage of herbarium specimens should not be considered as altogether final, especially where the collector simply endeavors to assign as nearly as possible a specific name to the host as the result of a general demand for such a name on the label.

The host is frequently given as *R. strigosus* in herbarium specimens of these rusts when it is clear that the host is a blackberry. There are seven packets of orange-rust in the Pathological Collections of the United

States Department of Agriculture, labeled to indicate that the host is *R. strigosus*. In every case except one the host is not a red raspberry. Nos. 277, 278, Ellis, N. Am. Fungi, are among this list. In No. 1014, Herbarium H. S. Jackson, the host which here bears only the telial stage is certainly either *R. strigosus* or *R. neglectus*, which is now recognized as a hybrid, the parents being *R. occidentalis* and *R. strigosus*. Specimens sent to the writer also proved that Rubus hosts are often confused. Where the host has been identified as red raspberry by the collector it is usually a blackberry.

LITERATURE CITED

(1) ARTHUR, J. C. Orange rusts of *Rubus*. Bot. Gaz. **63**: 501–515. *1 fig.* 1917. Bibliographical footnotes.

(2) ————AND G. R. BISBY. An annotated translation of the part of Schweinitz's two papers giving the rusts of North America. Proc. Amer. Phil. Soc. **57**: 173–292. *1 fig.* 1918.

(3) ATKINSON, GEORGE F. Selected cycles in *Gymnoconia peckiana*. Amer. Jour. Bot. **5**: 79–83. 1918.

(4) CLINTON, G. P. New or unusual plant injuries and diseases, found in Connecticut, 1916–1919. Connecticut Agric. Exp. Sta. Bull. 222: 397–482. 1920.

(5) DAVIS, J. J. Notes on the parasitic Fungi in Wisconsin. VI. Trans. Wisconsin Acad. Sci. **19**: 705–715. 1919.

(6) DODGE, B. O. Studies in the genus Gymnosporangium—I. Brooklyn Bot. Gard. Mem. **1**: 128–140. *5 fig. pl. 1.* 1918. Bibliographical footnotes.

(7) KUNKEL, L. O. Further data on the orange-rusts of Rubus. Jour. Agric. Res. **19**: 501–512. Pl. D (col.) pl. 92–93. 1920. Literature cited, p. 512.

(8) MICHAUX, ANDREAS. Flora Boreali-Americana. Vol. 1. 330 p., 29 pl. 1803.

(9) SAPPIN-TROUFFY. Recherches histologiques sur la famille des Urédinées. Le Botaniste **5**: 59–244. *70 fig.* 1896. Bibliographical footnotes.

(10) SCHWEINITZ, LEWIS DAVID VON. Synopsis fungorum Carolinae superioris.... Ed. a D. F. Schwaegrichen. 105 p., *2 col. pl.* [1822]. E. Commentariis, Societates naturae curiosorium lipsiensis excerpta.

(11) ———— Synopsis fungorum in America Boreali. Trans. Amer. Phil. Soc. n. ser. **4**: 141–316. *Pl. XIX.* 1832.

A BACTERIAL DISEASE OF BROME-GRASS

CHARLES S. REDDY AND JAMES GODKIN[1]

WITH PLATES I AND II

INTRODUCTION

In 1916, Professor L. R. Waldron, of the North Dakota Agricultural Experiment Station, called the attention of the senior author, then stationed at that place, to an apparently new disease of brome-grass (*Bromus inermis* Leyss.). The symptoms suggested that it was caused by bacteria, and subsequent isolations tended to confirm this. No fungi were found, and bacteria were readily isolated by methods herein described. On account of pressure of other duties, no inoculations were tried at that time. In the fall of 1919, the authors took up the problem. The investigations, extending over a period of three years, have been conducted at the University of Wisconsin and at Bloomington, Illinois. Numerous isolations and spray inoculations in the greenhouse have proved that the disease is caused by a colorless bacterium pathogenic to brome grass.

DESCRIPTION

In the early stages the disease appears on the leaves of *Bromus inermis* as light olive green, circular to elliptical water-soaked areas with light brown centers. Later the spots become dark chocolate or purplish brown and appear almost black. Old spots are usually linear, often coalescing to destroy the entire leaf surface of the infected plants. In early stages the spots are characterized by faded green to yellowish-green halos, which become more definite in the older spots.

In severely attacked plants, the upper nodes sometimes are killed by secondary infections of the organism. In these plants the panicles wither and die, producing symptoms similar to frost injury.

The seed may become infected by coming into contact with lesions on the upper leaves at the time the panicle emerges from the boot, or later the panicle may be blown by the winds so as to come into contact with leaf lesions. Also, splashing rains cause the disease to spread to the heads, as well as to other parts of the plant.

[1] These investigations were conducted at the University of Wisconsin during 1919, 1920, and 1921 under the direction of Professor L. R. Jones.

OCCURRENCE AND HOST RANGE

Each year from 1917 to 1921, the disease has been collected in the vicinity of Fargo, North Dakota, on *Bromus inermis*. In April, 1921, the disease, exhibiting its characteristic symptoms, was found on *B. inermis* at Madison, Wisconsin. In 1915, the senior author collected a bacterial disease on quack grass (*Agropyron repens* (L.) Beauv.) which exhibited symptoms similar to the brome-grass disease. A second collection on quack grass was made in company with A. G. Johnson in the fall of 1919 from which a colorless bacterium was isolated and proved pathogenic on *B. inermis*. As stated later, the brome-grass organism was found to be pathogenic on quack grass. Although no exhaustive comparison has been made between the two organisms, it is probable that they are identical. A spot on yellow foxtail is very common in the vicinity of Bloomington, Illinois. Since a bacterial disease had been reported previously on this host by Rosen (4), isolations were attempted. No bacterium could be found associated with these lesions, and later, inoculations with the brome-grass organism on yellow foxtail gave negative results. Natural infections on other hosts have not been observed although artificial inoculations have materially increased the host range.

ISOLATION OF THE ORGANISM

The blackened areas of the lesions were found to contain the bacteria in large numbers. The method most commonly used in making isolations was first to wash an infected leaf in clean, running water and then to cut pieces, about the size of the head of a pin, from the blackened areas of the lesions. Each of these pieces then was placed in a drop of sterile water previously placed in the center of a sterile petri dish by means of a flamed 4-mm. platinum loop. The tissue pieces were then mashed with a sterile instrument. When thinly seeded plates were desired, a small loop from each of the drops containing mashed tissue was transferred to each of another set of sterile petri dishes. Plain nutrient- or potato-dextrose agar then was poured into each of these in the usual manner and the dishes tipped back and forth in the usual way to distribute the bacteria uniformly. Colonies appeared in 36 to 48 hours and within three days after plating, streaks from single colonies were made on agar slants.

Isolations were made from both fresh and dried material. Isolations, from which the first artificial infections were obtained, were made from herbarium material, six months old, collected by the senior author

while at the North Dakota Agricultural Experiment Station. Subsequent isolations of the brome-grass organism have been made from this material up to 33 months after collection.

INOCULATION EXPERIMENTS

On Bromus inermis

The pathogenicity of each isolation was determined by inoculating *Bromus inermis*. At first 2 methods were used, wounding and spraying. The latter method gave more typical results, hence it alone was used in most of the later experiments. This method was essentially as follows: Sterile water was poured into tubes containing 2- to 5-day old agar streak cultures of the organism, and the bacterial growth was then allowed to diffuse into it. This suspension of the organism was applied by means of atomizers to the plants of various ages grown in pots. These were placed in damp chambers or covered with shaded bell jars for about 48 hours. The ends of the first leaves are very susceptible to infection and in 2 to 4 days turn black and wither. The more typical lesions appear in from 3 to 9 days on any parts of the leaves as small water-soaked infection points each with the indication of a halo about it. The water-soaked spots enlarge, gradually become darker until they are purplish black, and the halo, although usually not extending itself very far, becomes more distinct.

On Other Species of Bromus

A number of species of Bromus were grown in 4-inch pots in the greenhouse and, by means of atomizers, were inoculated without wounding when the plants were about 4 inches high. Both the brome-grass organism and the halo-blight organism (*Bacterium coronafaciens* Elliott) from oats were used. The results are presented in table 1. The plants in two pots of each species held as controls remained free of the disease. The degree of infection for each species in each experiment is indicated in the table as follows: 0 = no infection; 1 = slight infection; 2 = moderate infection; 3 = heavy infection.

All the species of Bromus inoculated were more or less susceptible to infection by the brome-grass organism. The resulting lesions in all cases, except on the five species noted below, were similar to those previously described for *Bromus inermis*. On *Bromus brizaeformis* Fisch. and Mey., *Bromus japonicus* Thunb., *Bromus molliformis* Lloyd, *Bromus tectorum* L., and *Bromus tectorum nudus* Klett and Richter, the centers of infection did not become black but were small, sunken, and

light gray with somewhat larger halos, resembling to a marked degree the halo-blight of oats. Of the species of Bromus inoculated with *Bacterium coronafaciens*, lesions developed on *Bromus carinatus* Hook. and Arn. only. These were typical "halo-blight" lesions with dead centers and large halos.

TABLE 1

Results of inoculations of species of Bromus with the brome-grass organism and Bacterium coronafaciens

Host	Brome-grass organism			*Bacterium coronafaciens*
	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4
B. arenarius Labill	1			0
B. brizaeformis Fisch. and Mey	2			0
B. carinatus Hook. and Arn	2	1	1	1
B. carinatus linearis Shear	2			0
B. commutatus Schrad	1			0
B. erectus Huds	2			0
B. hordeaceus L.	2	2		0
B. hordeaceus L. (Madison)	2	2	2	
B. inermis Leyss. (Arlington)	1	1		0
B. inermis Leyss. (Madison)	3	3	3	0
B. japonicus Thunb	1	1		0
B. lanuginosus Porr	1			0
B. marginatus Nees	1			0
B. molliformis Lloyd	1			0
B. mollis L.	1			0
B. pratensis Lam	2			0
B. richardsonii Link	3	3		0
B. rubens L.	2			0
B. secalinus L.	2			0
B. secalinus L. (Madison)	2	2	2	
B. sitchensis Bongard	1			0
B. sterilis L.	2			0
B. tectorum L.	2			0
B. tectorum L. (Madison)	2	2	2	
B. tectorum nudus Klett and Richt	1			0
B. unioloides (Willd.) H. B. K.	3	3		0
B. villosus Forsk	2			0

On Other Grasses

In six series of artificial inoculations made in the usual way the pathogenicity of the brome grass organism was tested on the common cereals and certain grasses. In each series brome-grass (*Bromus inermis*) be-

came moderately to heavily infected, quack grass (*Agropyron repens*) became slightly to moderately infected, and in two series oats became slightly infected. In all the series the results were negative on wheat (*Triticum aestivum*, L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), bluegrass (*Poa pratensis* L.), red top (*Agrostis palustris* Huds.), orchard grass (*Dactylis glomerata* L.), corn (*Zea mays* L.), timothy (*Phleum pratense* L.), yellow foxtail (*Chaetochloa lutescens* [Weigel] Stuntz), and barnyard grass (*Echinochloa crus-galli* [L.] Beauv.). In all, *Bromus inermis* has been inoculated approximately 24 different times and always with positive results, and the organism reisolated repeatedly.

In addition to the experiments referred to above, several parallel cross inoculations were made on brome-grass and oats, using both the brome-grass organism and the halo blight (*Bacterium coronafaciens*) from oats. Both organisms infected oats, but the lesions were not identical. Those caused by the brome-grass organism had a more water-soaked appearance and narrower halos than those caused by *B. coronafaciens*. *Bacterium coronafaciens* failed to infect brome-grass in these inoculation experiments, while, at the same time, good infections were secured with the brome-grass organism. As will be seen later, it is mainly on the basis of these cross inoculation experiments that the authors have felt justified in considering the brome-grass organism a variety of *B. coronafaciens* rather than identical with it.

CULTURAL CHARACTERS OF THE ORGANISM

This organism resembles *Bacterium coronafaciens* (3) except for minor differences. Hence, it seems necessary to compare them in only a few pertinent points and to present any additional studies made upon the brome-grass organism.

Bacterium coronafaciens Elliott from oats	Brome-grass organism
1. Average size 0.65 by 2.3 μ when stained with Ribbert's capsule stain, gentian violet, and carbol fuchsin.	1. Average size 0.72 by 1.92 μ when stained by aqueous gentian violet.
2. Thermal death point 47° to 48° C. Growth at 0° C.	2. Thermal death point 50° to 51° C. No growth at 0° C.
3. Killed by desiccation on cover glasses within 24 hours.	3. Remained alive in dried condition on cover glasses up to 198 hours.
4. Facultative anaerobe—growth in closed arms of tubes containing saccharose, glycerine, mannit, levulose, and dextrose.	4. Strict aerobe.

Bacterium coronafaciens Elliott from oats	Brome-grass organism
5. Good growth in 0.1 per cent citric, tartaric, and malic acid.	5. No growth in 0.1 per cent citric, tartaric, and malic acid.
6. No clouding in neutral beef-peptone bouillon containing more than 2 per cent sodium chlorid.	6. Clouding in neutral beef-peptone bouillon up to and including 7 per cent sodium chlorid.

Excluding infection experiments, the above statements present the differences in the morphology and physiology of the brome-grass organism compared with the morphologic and physiologic characteristics of *Bacterium coronafaciens* as given by Miss Elliott.

Realizing that these differences might be due to one or more of a number of factors, a culture of the oat organism was obtained from Miss Elliott and some of the experiments hitherto showing unlikeness of the two were repeated using both organisms side by side.

When the bacteria from 24-hour old cultures on plain nutrient agar were stained with aqueous gentian violet, the brome-grass organism averaged 0.72 by 1.92 μ, and the oats organism averaged 0.69 by 1.95 μ. The brome-grass organism from 7-hour old cultures on the same medium averaged 0.81 by 2.25 μ. All of these averages are measurements of 100 organisms in each case. Taking into consideration the possible variations in size, the two organisms can be considered identical morphologically.

Since it has been shown by Wolf and Foster (6) that, with plant parasites, variation in the reaction of the bouillon may cause the thermal death point to vary as much as 4° to 6° C., and since it is likely that other factors have as much effect, it was thought unnecessary to repeat the thermal death point experiment because the thermal death points already checked within 3 degrees.

Desiccation on cover glasses was not considered an important point of difference. The experiment was performed twice, using the brome-grass organism and growth occurred up to 175, and 198 hours respectively.

Growth occurred in shake cultures of plain nutrient agar at or near the surface only. No growth occurred in the lower end of stab cultures of plain nutrient agar or gelatin. No growth occurred in Roux anaerobic tubes filled with inoculated melted agar except at the ends of a few in which the agar did not completely fill the tubes. No increase in growth occurred in cultures placed in Novy anaerobic jars. At first growth was not consistent in the closed arm of fermentation tubes containing the various carbon compounds which was doubtless due to daily examination which necessitated handling the tubes. In this process a small

amount of the copious growth in the open arm was probably transferred to the closed arm and upon distribution gave the closed arm the appearance of being slightly clouded. When both organisms were used in a complete fermentation experiment and half of each set was left undisturbed for 17 days, there was no growth in the closed arms of the undisturbed tubes. This was true in the case of those inoculated with *Bacterium coronafaciens* as well as in the case of those inoculated with the brome-grass organism. In the tubes that were handled each day, part of them showed clouding in the closed arms. These results indicate that, although the two organisms are identical in this characteristic, they are obligate aerobes rather than facultative anaerobes.

Both organisms were grown in bouillon (pH = 6.8) containing various percentages of sodium chlorid up to 8. Both organisms clouded bouillon up to and including that containing 7 per cent sodium chlorid.

TECHNICAL DESCRIPTION

Bacterium coronafaciens Elliott var. **atropurpureum** n. var.

Variety agrees with the species in morphological and cultural characters. Differs in appearance of lesions and host range. Lesions on type host, *Bromus inermis*, have moderately developed to no halos, and oval to linear, purplish black centers 1 to 5 mm. in diameter. Artificial inoculations of *Avena sativa* produce similar lesions except that the centers are translucent not dark. In contrast, lesions on oats caused by *Bacterium coronafaciens* have small, brown, dead centers surrounded by large halos. Hosts are *Bromus inermis*, *Bromus carinatus*, numerous other species of Bromus, oats, and quack grass (*Agropyron repens*). In contrast the hosts of *Bacterium coronafaciens* are oats, *Bromus carinatus*, wheat, rye, and barley.

Habitat.—North Dakota and Wisconsin.

ADDITIONAL EXPERIMENTS ON THE PHYSIOLOGY OF THE ORGANISM

It is thought probable that, with two bacterial organisms which are almost alike except as to infectivity, extended physiological experiments would show distinct differences, if carried far enough, especially along cultural lines. With this in view, the following investigations were conducted with *Bacterium coronafaciens* and *B. coronafaciens* var. *atropurpureum*. Although no further definite differences were discovered, certain additional data on the two organisms were obtained.

Indicator Media

Saccharine media containing mixtures of brom cresol purple and cresol red were prepared according to recommendations in the "Report of the Committee on Descriptive Chart" (2). Eight agar slants of each medium were inoculated with the brome-grass organism and eight of each with *Bacterium coronafaciens*. Both organisms changed the color to the same degree in the same length of time in each medium. Acid reaction was produced in the media containing dextrose, or saccharose as carbon compounds. Alkaline reaction was produced in those media containing lactose, maltose, mannit, or glycerine. Controls were unchanged.

Fermentation Tubes

One per cent peptone water (Dunham's solution) with 2 per cent respectively of dextrose, saccharose, maltose, lactose, mannit, and glycerine were used to make up the carbon compounds. Nitrate bouillon and milk also were used. Good growth was obtained in the open arm of all tubes by inoculation with the brome-grass organism. At the end of 30 days no gas was produced and no growth occurred in the closed arms. Table 2 shows the Fuller scale and pH readings taken during the course of the experiment.

TABLE 2

Reactions of different media before and following inoculation with Bacterium coronafaciens var. atropurpureum

Media	Reaction before inoculation	Reactions following inoculation		
		20 days	30 days	50 days
Dextrose	(Fuller's scale = + 6.5	+15.0	+18.0	———
	(pH values = 6.5	4.9	4.4	———
Saccharose	(Fuller's scale = + 6.0	+15.5	+16.0	+17.5
	(pH values = 6.6	5.0	4.9	4.9
Maltose	(Fuller's scale = + 7.5	+ 5.0	+ 3.5	+ 3.0
	(pH values = 6.8	8.2	8.4	8.5
Lactose	(Fuller's scale = + 4.0	+ 3.0	+ 1.0	———
	(pH values = 6.6	8.4	8.4	———
Mannit	(Fuller's scale = + 4.0	+10.0	+ 3.0	———
	(pH values = 7.0	8.2	8.4	———

TABLE 2—*Continued*

Media	Reaction before inoculation	Reactions following inoculation		
		20 days	30 days	50 days
Glycerine	(Fuller's scale = +10.5	+ 6.0	+ 3.5	+ 3.0
	(pH values = 6.6	6.6	7.0	8.4
Milk	(Fuller's scale = + 9.0	+ 6.5	+ 7.0	+13.0
	(pH values = 6.7	7.39	7.55	7.2
Nitrate bouillon	(Fuller's scale = +10.0	+ 5.0	+ 3.0	——
	(pH values = 7.0	8.2	8.5	——

Reduction of Nitrates

As the organisms produced ammonia, probably from peptone, when grown in bouillon, no final conclusions could be reached from the first experiments in which ordinary nitrate bouillon was used. The organisms were then grown in a synthetic medium containing small quantities of phosphate, calcium, chlorine, etc., with KNO_3 as a source of nitrogen and sucrose as a source of carbon (2). Good growth on this medium was obtained. Tests with sulphanilic reagent for nitrites and Nessler's reagent for ammonia gave negative results. These results, together with the fact that no gas is produced in fermentation tubes containing nitrate bouillon, lead to the conclusion that nitrates are not reduced.

Optimum Reaction and Toleration Limits

Beef peptone bouillon was adjusted to each of the following reactions with sodium hydroxide, and hydrochloric acid + 27 (pH = 3.8), + 17 (pH = 4.8), + 12 (pH = 5.2), + 5 (pH = 7.2), 0 (pH = 8.6), — 4 (pH = 9.0), — 10 (pH = 9.1), — 15 (pH = 9.6), — 20 (pH = 9.8), — 30 (pH = 9.9).

Optimum reaction for growth + 5 (pH = 7.2). Limits of growth were + 12 (pH = 5.2) and — 15 (pH = 9.6).

Susceptibility to $CuSO_4$ and $HgCl_2$. The experiments were run in triplicate and the average results presented in table 3.

TABLE 3

Effect of $CuSO_4$ on Bacterium coronafaciens var. atropurpureum

Dilutions expressed as ratio of $CuSO_4$ to H_2O	Number minutes treated	Number of colonies developing in petri dishes containing 10 cc. of nutrient agar inoculated with the following amounts of the various $CuSO_4$ dilutions, in 10 cc. portions of which had been placed a uniform amount of inoculum		
		4 mm. loop	3 mm. loop	1 needle
0 (Control)	5	487	375	5
0 (Control)	10	563	450	8
0 (Control)	15	750	562	14
1:50,000	5	0	0	0
1:50,000	10	0	0	0
1:50,000	15	0	0	0
1:100,000	5	5	0	0
1:100,000	10	0	0	0
1:100,000	15	0	0	0
1:500,000	5	163	0	0
1:500,000	10	16	0	0
1:500,000	15	5	0	0

In a similar experiment, using mercuric chlorid in place of copper sulphate, the numbers of colonies developing in the controls were practically the same as recorded for the controls in table 3. No colonies developed in the remainder of the experiment except an average of 9 in the 1:500,000 dilution using the 4-mm. loop and allowing an exposure of 5 minutes.

Brome-grass Organism in Relation to Host Tissues.

Infection occurs through wounds and stomata. Spraying unwounded leaves of *Bromus inermis* with a water suspension of the organism results in numerous infections. A study of razor sections of young lesions obtained in this way shows that infection may be stomatal and is at first intercellular. Studies of stained sections of diseased and normal tissues which were killed in absolute alcohol and embedded in paraffin show that in old lesions the cells are misshapen, disrupted, and usually crowded full of bacteria. It is probably because these parenchymatous cells are so completely filled with bacteria at this stage that the lesions

do not present a collapsed appearance with sunken areas as is the case with the lesions of Halo-blight of oats.

Overwintering and Dissemination of the Brome-grass Organism

No special work has been done on this phase of the problem. Isolations from old material, together with field observations on the recurrence of the disease each year at the same places, lead to the belief that the organism overwinters in the lesions on the dead brome-grass. It is suspected that this disease is transmitted by the seed.

SUMMARY

1. The bacterial blight of brome-grass sometimes produces spots on the glumes and causes abundant dark purplish-brown spots on the leaves. The spots may or may not be accompanied by yellow halos. The upper nodes are occasionally killed.

2. Natural infections have been found on *Bromus inermis* and *Agropyron repens*.

3. Artificial inoculations show that the white bacterial organism found constantly associated with the disease is capable of infecting 23 species of the genus Bromus, oats (*Avena sativa*), and quack grass (*Agropyron repens*).

4. The organism isolated by the writers has the group number 211.-2323023. The same number is suggested as the correct number for *Bacterium coronafaciens*.

5. *Bacterium coronafaciens* in several experiments produced no infection on *Bromus inermis*. The lesions produced on oats by the two organisms are not identical.

6. On account of the differences in infectivity between *Bacterium coronafaciens* and the brome-grass organism, the latter is considered a variety of the former and given the name *Bacterium coronafaciens* var. *atropurpureum*.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.
in cooperation with
WISCONSIN AGRICULTURAL EXPERIMENT STATION,
MADISON, WISCONSIN.

LITERATURE CITED

(1) CONN, H. J. AND G. J. HUCKER. The use of agar slants in detecting fermentation. New York Agric. Exp. Sta. Tech. Bull. **84**: 3–4. 1921.

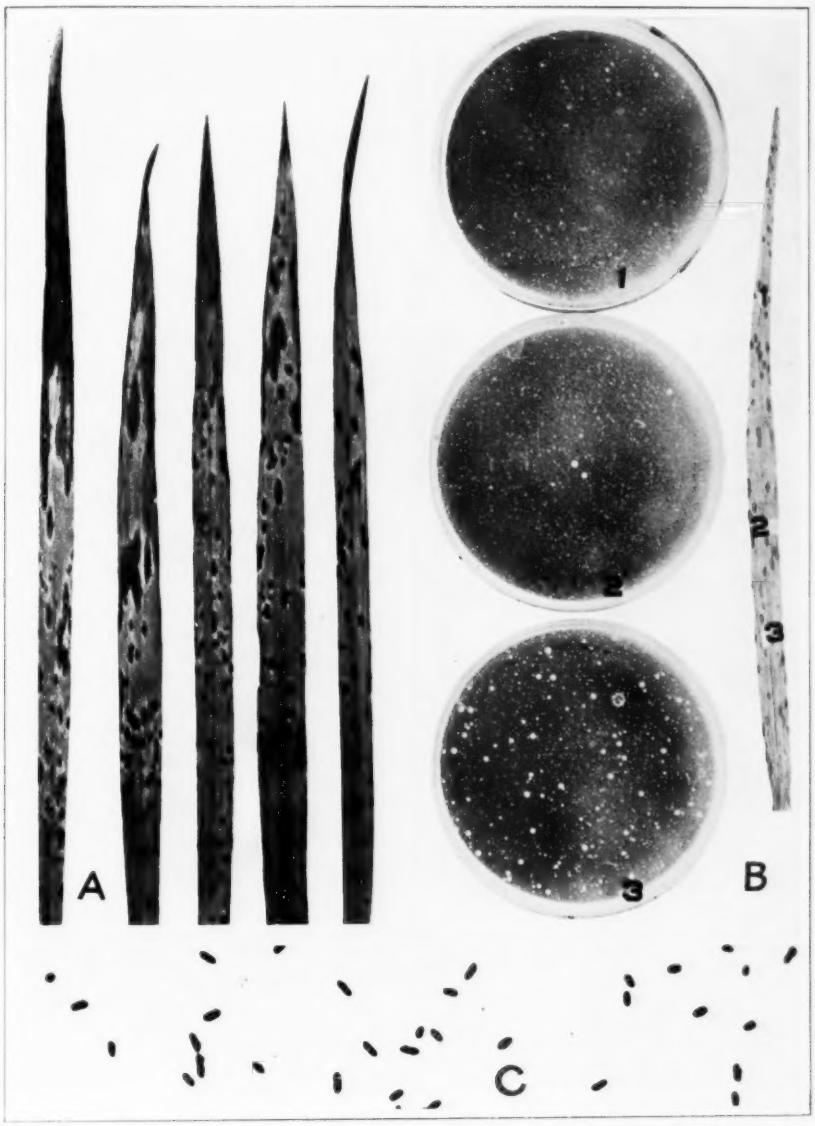
(2) ————et al. Report of the committee on the descriptive chart for 1919. Jour. Bacteriology **5**: 127–143, 315–319. 1920.

(3) ELLIOTT, CHARLOTTE. Halo-blight of oats. Jour. Agric. Res. **19**: 139–172. *Pl. 26–34*, pl. C (col.). 1920.

(4) ROSEN, H. H. A preliminary note on a bacterial disease of foxtail. Science n. s. **49**: 291. 1919.

(5) SMITH, ERWIN F. Bacteria in relation to plant diseases. Vol. 1, xii, 285 p., 146 fig., 31 pl. Washington, D. C., 1905. (Carnegie Inst. Washington Publ. 27.)

(6) WOLF, FREDERICK A. AND A. C. FOSTER. Studies on the physiology of some plant pathogenic bacteria. North Carolina Agric. Exp. Sta. Tech. Bull. **20**: 21–24. 1921. Literature cited, p. 24.



BACTERIAL DISEASE OF BROME-GRASS

A. Typical bacterial lesions on leaves of brome-grass (*Bromus inermis*) from natural infection. Marginal halos vary from moderate to none. B. Colonies of the organism (*Bacterium coronafaciens* var. *atropurpureum*) in first plates of isolations from leaf lesions on brome-grass indicated by corresponding numbers. The methods of isolation are described on page 76. C. The organism (*Bacterium coronafaciens* var. *atropurpureum*) stained by Zettnow's method to show flagella.

BACTERIAL DISEASE OF BROME-GRASS

Lesions produced by spraying a water suspension of *Bacterium coronafaciens* var. *atropurpureum* on unwounded leaves of: A. *Bromus inermis*, B. *Agropyron repens*, C. *Bromus secalinus*, and D. *Bromus hordeaceus*.

EXPERIMENTS WITH PARIS DAISY AND ROSE TO PRODUCE RESISTANCE TO CROWN GALL

NELLIE A. BROWN

WITH PLATES III AND IV AND FOUR FIGURES IN THE TEXT

Our early work with the crown-gall organism was carried on largely with the Paris daisy, and as the facilities were favorable for some studies on resistance, an experiment was started in the year 1907 with this end in view.

The plan devised was to start with daisy plants which were perfectly healthy, to inoculate them with the crown-gall organism and when the galls were full sized and the wood of the plant in condition for making cuttings, to remove slips from these galled plants. When the cuttings were full-sized plants, they were to be inoculated with the same strain of *Bacterium tumefaciens* or a strain known to be very infectious, and so on through a number of generations with the hope that resistance would eventually develop.

The work was carried through in two series of experiments, one extending from 1907 to 1912 which included 7 sets of cuttings, and 8 sets of inoculations; but of these no group photographs were made; the second experiment extending from 1912 to 1917 with 5 sets of cuttings each of which had been galled by pure culture inoculation. (Pls. III and IV.) The method used was needle prick inoculations throughout the experiment.

The details of the experiments are as follows:

FIRST SERIES OF EXPERIMENTS WITH PARIS DAISY

March 8, 1907. Twelve healthy Queen Alexandra daisy plants, propagated from cuttings, were inoculated with *Bacterium tumefaciens*. Galls formed readily and were 1.5 to 2 inches in diameter in two months. Cuttings were then made from these plants and in July when well-rooted and growing vigorously they were inoculated. Large galls formed at once and in November a set of cuttings was made from these plants. The November cuttings did not grow well during the winter but started a rapid growth in the spring and were inoculated in April, 1908. For some reason the galls were slow in forming and as the plants were in

good condition it was thought perhaps some resistance to the organism had developed. It was August before the galls were of any appreciable size; cuttings were rooted from these galled plants and were inoculated in November, 1908.

Whenever cuttings from diseased plants were inoculated other plants of the same variety (the Queen Alexandra daisy) were inoculated as controls. The controls were selected from plants which had never before been inoculated.

The plants inoculated November 18, 1908, which were expected to produce galls of the 4th series in line of successions from the original plants, failed to produce galls in the usual time. The control plants growing under similar conditions had galls in less than a month. This 4th series of plants was inoculated again, but even nine months afterwards there were no galls at the inoculated places. Suspecting immunity was becoming established, cuttings were made from these plants in March, 1909. (5th set.) In May, 1909, these new cuttings were in good condition for inoculating. The same old strain of *Bacterium tumefaciens* used before was used again, also another strain isolated from a peach gall.

In July, 1909, galls had formed on the controls whether inoculated with the old daisy strain or with the peach strain, but not on the plants in the series with either strain. This showed that the old daisy strain was still infectious and the circumstance seemed to point again to the establishment of resistance.

Next a new and very infectious peach strain which had been tested out was tried on the suspected resistant cuttings. No galls formed on them (16 in all) while control daisies of a new Queen Alexandra stock were readily infected.

On December 3, 1909, the 5th set of cuttings was tried a third time. Thirty-one plant cuttings of this series were inoculated with the daisy strain; as controls 6 young Queen Alexandra daisy plants never before inoculated were used, also 8 sugar-beet plants.

December 28, 1909. The control daisy plants had galls an inch in diameter and two months later the control sugar beets had galls 3 to 4 inches across, but there was only one daisy of the cutting series with a gall.

January 18, 1910. In the 5th series inoculations made December 3, 1909, several of the plants which showed negative results after 30 days were now producing galls. In two more months all of the 31 plants had galls of varying sizes. These plants had some resistant tendency which

was overcome as growth continued. Such resistance had never occurred before,—galls usually started on young growing plants in from one to two weeks after needle prick inoculations.

Cuttings were made in the spring of 1910, from the last (5th) set of galled plants. The 6th set in the series did not get into condition for inoculating until winter. The organism used for inoculating was an isolation from a gall on a plant of the 5th set of cuttings; its virulence had been proved on other plants (the supposition at the time of isolating was that the colonies would not be infectious but this one colony proved to be more virulent than any strain from the Paris daisy previously isolated).

On January 12, 1911, plants of the 6th set of cuttings were inoculated with this virulent strain. It was named the "Resistant Daisy" strain, to distinguish it from other daisy strains in use, as it was isolated from a gall on a plant which for a time according to appearances had acquired resistance. This strain is still in use (1921) and is still virulent, while the organisms of other earlier and later daisy isolations have weakened or lost their virulence entirely. This strain (Resistant daisy) inoculated into the 6th series of cuttings produced galls rapidly, of a large size and for the first time in the course of the experiment secondary galls were formed on stems and leaves (Fig. 1). This affected the plants badly in growth and looks. The control inoculations which were made on new plants, purchased in Boston and Springfield, had large galls also, but no secondary galls. The galls started to form more quickly on the controls than on the plants of the series test.

Cuttings were made from the plants with secondary galls, (6th set) in the summer of 1911, making the 7th set from galled plants. The growth of these cuttings was slow,—the plants seemed not to be vigorous in the fall and it was not until January 7, 1912, that they were inoculated. At this time they had started a good growth. This was also true with the 6th set of cuttings, the slips of which were slow to take root and slow in getting a good growth started. At the time the 7th series was inoculated (Jan. 7, 1912), which included 27 plants, controls were held on other new Queen Alexandra daisy plants from Boston, never before inoculated. As was true with the 6th series, galls formed more quickly on the control plants but ultimately were no larger than the galls in the series test. The control plants themselves looked much stronger however than the plants in the series test. In fact, when it was time to make new cuttings from the 7th set, the plants were in such poor condition that none could be obtained. The plants continued to look weak

Fig. 1 Two Queen Alexandra Daisy plants belonging to the 6th set of cuttings in the first series test, inoculated with organism isolated from gall on previous set in series. Inoculations made January 13, 1911, with strain called Resistant Daisy. Photographed March 22 and 27, 1911, respectively.

all summer and in August all were dead but one. This plant, was set aside for later cuttings, but through a gardener's mistake it was discarded with the rest.

Discussion of results:—The death of the plants in the series test was rather a surprise, the full significance of which did not become clear until it had occurred again—namely that the constitution of the plants had become weakened through the continual production of tumors in successive generations of cuttings, so weakened that death followed.

In the six years that work had been carried on with galled daisy plants it had been found that they did not die from the presence of galls unless the galls encircled the stem. In former cases the plants were not galled in succession. The encircling stage was rather rare. The galls in the series test had not encircled the stem although the plants had died. The controls were alive and in fair condition with full sized galls on them. The conclusion therefore was that death of the series plants had resulted from a weakened condition due to a continual diseased state induced by the presence of galls on each of seven successive generations. And instead of a resistance to *Bacterium tumefaciens* being constitutionally established in the daisy plants an increased susceptibility had developed.

It remained for further tests to be made along this line to see if this susceptibility could be repeated.

SECOND SERIES OF EXPERIMENTS WITH PARIS DAISY

The second series of experiments was started in July, 1912, by making cuttings from galled control plants of the Queen Alexandra daisy, the plants never having been galled but this one time.

In January, 1913, the cuttings of this set were in good growing condition and the plants were inoculated January 7, 1913. Forty-nine plants were inoculated using the strain of *Bacterium tumefaciens* called "Resistant Daisy," which as before stated had been isolated from one of the 5th set of galled plants in the former "resistance test." In March, 1913, galls two inches in diameter had formed on the daisies. Cuttings were now made from these plants.

December 2, 1913, plants of the 2d set of cuttings (28 in number) were in good condition and were inoculated with *Bacterium tumefaciens* (3d set of inoculations with "Resistant daisy strain.")

February 3, 1914. Galls ½ to ¾ inches in diameter were on the plants. In May of the same year all of the twenty-eight plants inoculated had produced galls. Cuttings (3d set) were made from these plants in July.

December 15, 1914. Thirty plants of the 3d set of cuttings were inoculated. Controls were held on 13 daisy plants never before inoculated.

Galls were forming well on the series plants in February, 1915. In May, 1915, photographs were made of 27 plants of this set (galled 4 times) and also of 12 of the controls (Pls. IIIA, and IIIB: IVA and IVB). The photographs show a marked contrast. The series plants were stunted and some of them dying. Three of the 30 could not be found. They had in all probability died and had been thrown out by a gardener. The control plants, bearing galls for the first time, were in good condition.

In May, 1915, after photographing, cuttings were made from the plants which had been galled the 4th time. December 20, 1915, the resulting plants (22 in number) of this 5th set of cuttings were inoculated with the Resistant daisy strain of *Bacterium tumefaciens*.

Twenty Queen Alexandra daisy plants never before inoculated were inoculated for controls. These last were grown under the same conditions as the other plants. Galls formed rapidly on all. In a week small tumors were visible. In a month (January, 1916) all the plants, series and controls, had galls three-fourths of an inch in diameter. This made the 5th set of galls in the series. In May, 1916, cuttings were made. They did not root very readily and struggled along through the summer. In the fall they were all dead but one. This one was unhealthy but it improved somewhat during the winter of 1917, and in the spring cuttings were made from it. In June, however, all these cuttings of the 6th set were dead and in the fall the parent plant died also. The cuttings never developed well and so were not inoculated. The inoculations, therefore, of this series ended with the fifth.

Discussion of results:—The results of the second experiment were like those of the first—no decided resistance to *Bacterium tumefaciens* was developed by the successive formation of galls on cultivated daisy plants, the plants used being cuttings of infected plants in successive generations. What appeared to be resistance in one stage of series I being due, it is most likely, to decreasing virulence on the part of the cultures used; the series plants requiring a more virulent strain to infect them than did the control ones. The results, however, in both series seem to indicate that the vitality of the plants was considerably reduced by repeated infection with the organism. This fact was shown by the dying of the plants after they had been galled in the line of successive cuttings five to seven times, although the cuttings made from them were taken while the plants were still in good condition.

EXPERIMENTS WITH JUICES OF GALLED PLANTS

At one time during the first series experiment, when the fifth set of cuttings after inoculation gave the appearance that resistance to the crown-gall organism was either established or becoming established, a number of the full-grown plants of this set were brought to the laboratory and treated in the following way. Stems and leaves were washed care, fully, ground, and put through the pressure filter. Varying amounts-(1cc. to 5cc.) of this filtrate were added to tubes of liquid agar, the agar was then inoculated with the crown-gall organism and plates poured. The juice proved to have no permanently inhibiting effect, for typical colonies of *Bacterium tumefaciens* appeared on the plates in great numbers but when compared to control plates, made with juice from uninoculated plants, there was marked retardation in the growth of the colonies. The colonies were inoculated into healthy daisy plants, also into sugar beets. Galls formed, so the virulence of the organism was in no way reduced by the juice from the supposedly immune plants. Other experiments with the juice from infected plants were as follows:

1 to 3 cc. of the juice were added to beef agar slants, after they had been inoculated with *Bacterium tumefaciens*. Beef agar slants without the juice were also inoculated as controls. Growth was retarded in the slants with the juice, but once started the organism developed rapidly and was no less abundant than in the control plants.

To a bouillon culture of *Bacterium tumefaciens* 10 cc. of the diseased filtered juice were added also. Plates were poured from this treated culture using plain beef agar to which 3 and 5 cc. of diseased boiled juice were added to each tube. Likewise plates were poured and slant cultures were made with *Bacterium tumefaciens* using the juice of healthy daisy plants in the same way as the diseased plant juice. This juice was obtained in the same manner by passing the ground up plants through a pressure filter. Healthy daisy plants juice was also boiled without passing through the filter and then added to agar as in the test with the diseased plant juice.

Results:—The results showed that the organism grew in the media to which diseased daisy plant juice (either passed through the filter or boiled), and healthy daisy plant juice had been added respectively. No difference could be detected except in the time of the appearance of growth of the organism in the cultures. The organism in those cultures with diseased juice added was slower in making its appearance.

INOCULATIONS WITH DEAD CULTURES

An interesting experiment was one in which dead cultures of *Bacterium tumefaciens* were injected into the fibro-vascular bundles of daisy plants and the plants then inoculated in the internode above the injected area. Others on the other hand were inoculated in the spot in which dead cultures were injected. Galls formed on every stem in the former case in three weeks, but in the latter out of 11 plants inoculated only 2 produced galls and these did not appear until four weeks after inoculation.

That the culture was dead was proved by transferring some of it to bouillon and Uschinsky's solution. No development of the organism followed.

The details are as follows:

December 28, 1909. Bouillon cultures of *Bacterium tumefaciens* were heated to 60° C. for 10 minutes then injected after cooling into young Queen Alexandra daisies. About 3 cc. of the dead cultures were injected in each of 24 shoots. The needle was thrust downward in the fibro-vascular bundles. The next day another injection of a dead culture was made in the exact location as before. Immediately afterward 13 of the plants were inoculated by needle pricks in the internode just above the puncture made by injecting the dead culture while eleven of the stems were inoculated *in* the injection puncture.

Two plants not injected were inoculated as controls.

January 11, 1910, galls had formed on the controls. Three weeks after inoculation, galls were forming on internodes above the point of injection but none where inoculations were made in injection openings.

Forty-two days after inoculating two out of the eleven plants inoculated in the injected places had galls forming but these were very small in comparison to the control galls, or those on the plants where inoculations were made in the internodes above the injected areas. No further gall development occurred in the injected places

BREEDING EXPERIMENTS TO GET ROSE SEEDLINGS RESISTANT TO CROWN GALL

The rose was used in this problem because of the prevalence of rose galls in greenhouses where roses are grown commercially for cut flowers and also for the sale of rooted cuttings. The disease occurs out of doors also but not so extensively as in greenhouses. Most varieties of roses are susceptible to tumor formation but the condition of growth of the

plant (whether growing rapidly or partially dormant) is an important feature in the character of the infection which follows. Because of this it is difficult to learn definitely the varieties which are least susceptible; facts which were considered necessary so that crosses could be made with these varieties. Inoculations were made on twenty or more commercial varieties in the hope of finding some variety that would be immune. Oftentimes the inoculations failed to take and the variety was considered one suitable for crossing, it being suspected that it might be an immune variety or at least less susceptible than some of the others. However, when inoculations were repeated later to verify former tests the conclusion would be entirely overthrown by the appearance of a destructive gall. It remained then to use those varieties which seemed to possess resistance and by crossing them to follow up their progeny with the hope that some of the resultant seedlings would exhibit resistant tendencies. It might happen that some of the seedlings would even entirely resist infection. The crosses were made with those roses which were in favor commercially. As indicated above there was little chance of any constant inherent resistance that could be depended upon in making these crosses.

A virulent strain of *Bacterium tumefaciens* isolated from rose galls occurring in a Massachusetts rose-house was used for inoculation throughout the experiment (Fig. 2A). When galls had developed on the stem or preferably were starting to form on the pedicel of a flower, crosses were made with the following varieties: Mrs. Charles Russell, Sunburst, Ophelia, Irish Fireflame, My Maryland, Pink Killarney, White Killarney, Prince Charles d'Arenberg, Lady Alice Stanley, Red Radiance, September Morn, Hoosier Beauty. This was done in the spring of 1916 and 1917.

February, 1917, five rose hips were ripe on galled plants. These were cut off and planted. An accident happened to two of them but of the three that remained, crosses were as follows: Mrs. Charles Russell × Sunburst; Ophelia × Irish Fireflame; My Maryland × Ophelia. Both Irish Fireflame and Mrs. Charles Russell had been slow in producing tumors after inoculation; many inoculations failing entirely to take.

The seeds germinated September and October of 1917, and throughout the summer of 1918, there were ten struggling plants. A year later only five were alive, three of which were large enough to use for cuttings. Later the other two puny plants died. Thirty-six cuttings were made from three seedlings, nineteen of them being from one which produced

a double rose-red blossom (Mrs. Charles Russell × Sunburst;) two from a double cream-pink (My Maryland × Ophelia), and fifteen from a single cream-pink blossom (Ophelia × Irish Fireflame).

In April 1920, the plants were in good growing condition and were inoculated. Controls were held on stock rose bushes which had never

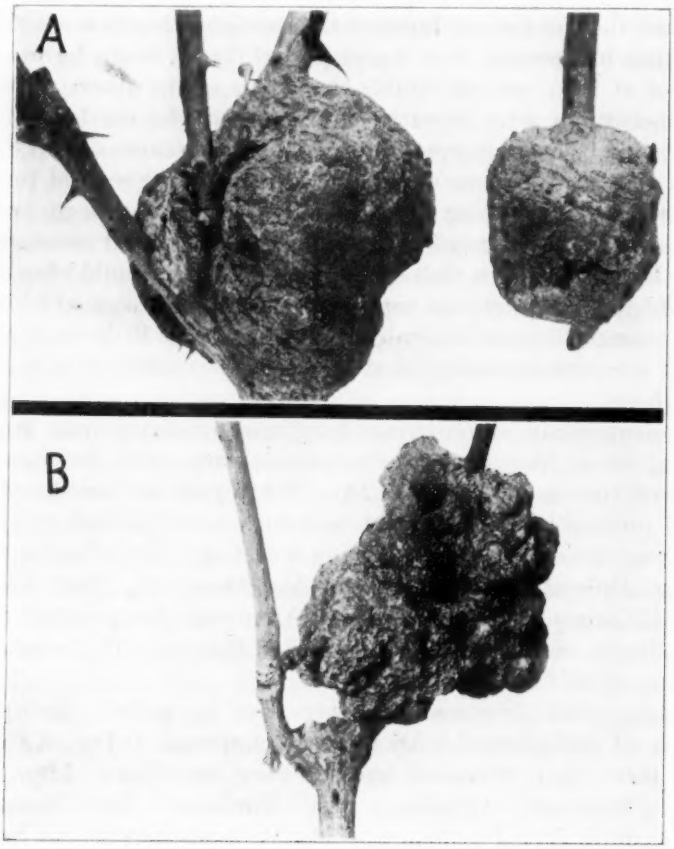


Fig. 2. A. Crown galls on rose stems from Waltham, Mass., April 14, 1915. Natural size. Isolations made from these.

B. Ophelia rosebush inoculated with rose gall organism June 2, 1920 (Fig. 2a). Photographed April 6, 1921. Plant had been dead two months. One of the controls in the hybrid resistance test which shows the virulence of the organism.

been inoculated. These were My Maryland, Ophelia, Prince Charles d'Arenberg, Sunburst, Irish Fireflame, and American Beauty. Controls were also held on cauliflower plants, sugar beets and Ricinus.

In one month after inoculating galls were forming on the single cream-pink bushes but none on the double cream-pink or the rose-red bushes. In two months there were small galls 1.5 and 4 cm. in diameter on 3 inoculated plants of the double cream-pink variety and one much smaller on the rose-red. By this time a single-pink seedling gall was 2 by 3 cm. (Fig. 3a) while the one rose-red seedling gall was only 3 by 4 mm. (Fig. 3b). Galls 2.5 to 4.5 cm. in diameter had formed on the cauliflower, sugar beets and Ricinus (Fig. 4). On the control rose bushes they were 2 to 2.5 cm. in diameter. More control inoculations were made in May and June to learn the type of virulence of the organism during the hot months.

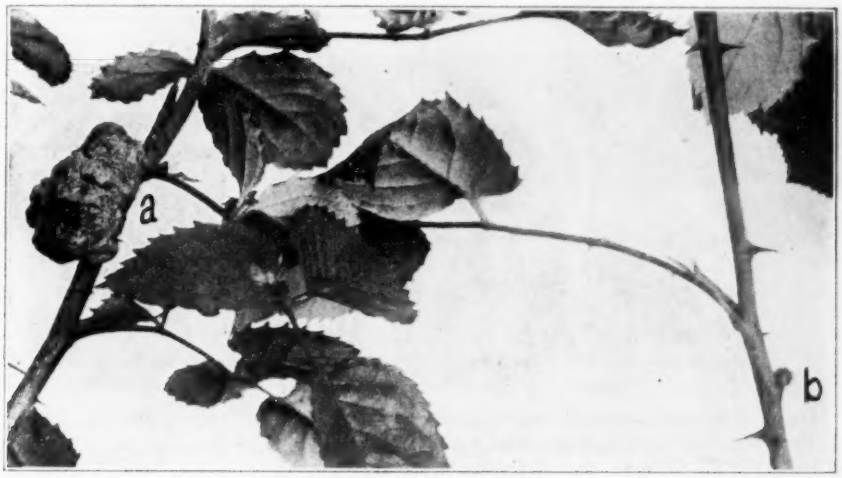


Fig. 3. Rose gall in hybrid immunity test. Seedling single cream-pink (Ophelia × Irish Fireflame) left; seedling rose-red (Mrs. Charles Russell × Sunburst) right. Both inoculations April 5, 1920, with same culture or rose-gall organism. Photographed June 18, 1920. *a*, gall 2 by 3 cm.; *b*, gall 3 by 4 mm. Natural size showing the temporary resistance of one variety to the crown-gall organism.

The results of the seedling inoculations at the end of July 1920, were as follows: Out of 19 rose-red bushes, (Mrs. Charles Russell × Sunburst) inoculated on many stems, 15 had no galls, even after three months; the other 4 had small galls 5 mm. to 1 cm. in diameter. Of the 15 single cream-pink variety (Ophelia × Irish Fireflame) only 8 had grown in a thrifty way, so that tender shoots could be inoculated. Six of these 8 produced galls 2 to 3 cm. in diameter, much larger than those of the rose-red variety. Of the two plants of the double cream-pink variety

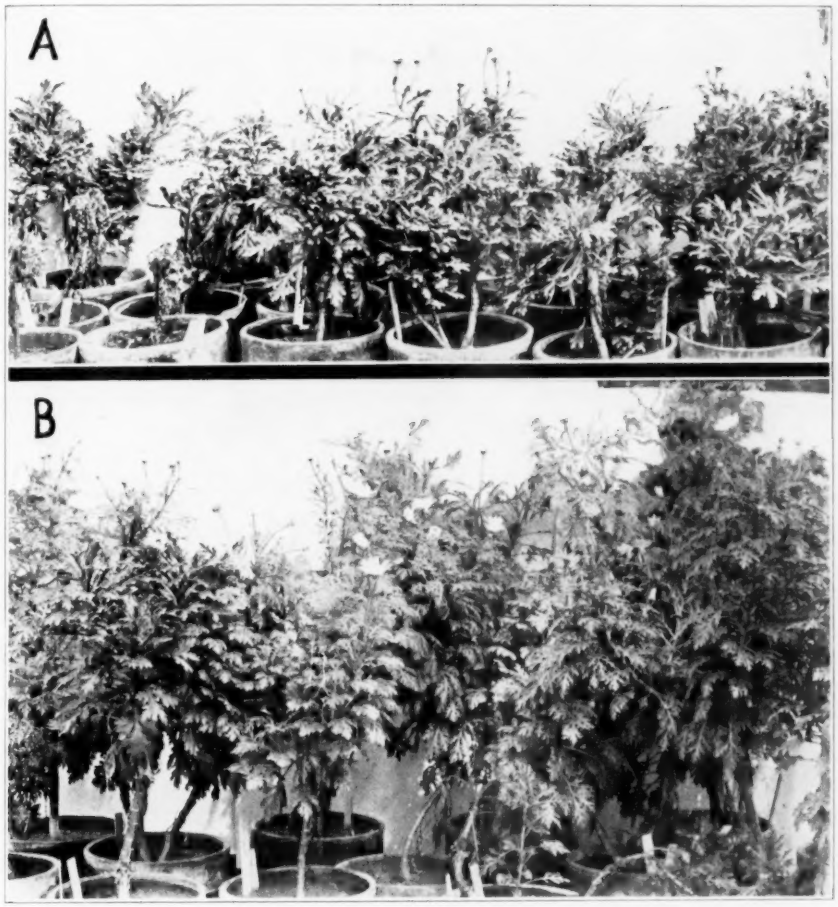
(My Maryland × Ophelia) one had two large galls 2 to 4 cm.; the other plant was not inoculated. Secondary galls were later produced on the single cream-pink variety. After a year had elapsed the rose-red variety had only one sizeable gall 3 cm. in diameter while two of the single pink variety had died from the galls encircling the stem. One of the control rose bushes (Ophelia) had also died, its stem being encircled by the gall (Fig. 2B). The other controls including several Ophelia bushes had galls of various sizes but were producing flowers occasionally.

In the spring of 1921, a year after the first inoculations were made into the rose seedlings, when the bushes were sending out new growth the new spring shoots were inoculated. Special attention being given to the rose-red variety. Twenty inoculations of the rose-red variety were

Fig. 4. Rose galls on sugar beets, cauliflower and Ricinus. Plants inoculated Apri 5, 1920, with the Rose-gall organism, to test its virulence and as a control on the supposed resistant bushes. Photographed June 18, 1920. Reduced in size.

made; no infections followed. Galls formed readily on the single Cream-pink variety. The plants of the double cream-pink were not growing well and were not inoculated.

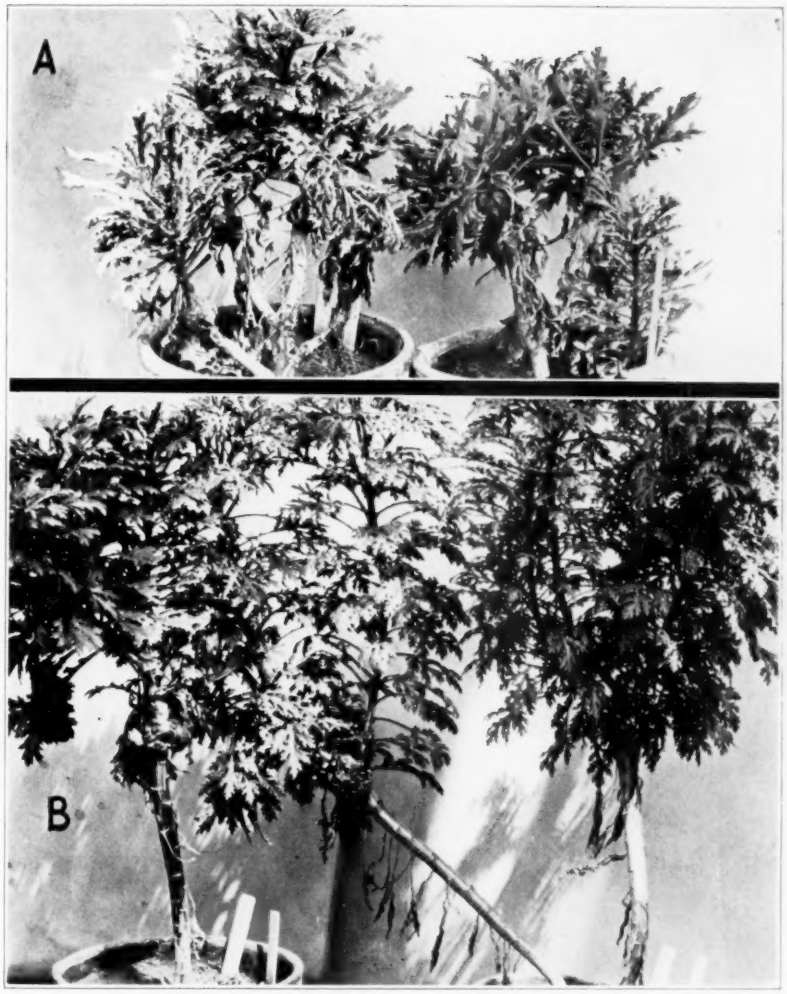
During June and July plants of the rose-red variety, (Mrs. Charles Russell × Sunburst) were inocluated again. This time galls formed in nearly 60 per cent of the inoculations showing that the resistant characters of this plant were not permanent. The galls were smaller and slower in growth than those of the two other varieties. However, they continued to increase in size during the winter of 1922 and although development was slow, in the spring they were as large as any mature galls on the two susceptible varieties.



CROWN GALL

A. Daisies in second immunity and susceptibility test. Galled 4 times. That is cuttings have been made from galled plants and their cuttings inoculated to the 4th series. These plants were inoculated with Daisy organism "Resistant strain" December 15, 1914. Photographed May 3, 1915. Seventeen out of 27 shown; print cut down.

B. Daisy plants inoculated with Resistant Daisy Colony 2, December 15, 1914. Photo. May 3, 1915. These plants are controls on the immunity and susceptibility test. They had never been galled before, that is, they are cuttings from plants which had never had galls. Two of these were photographed out from the group. There are galls on all. The photograph shows that the plants are in good condition after 5 months when galled only once. Same magnification as A.



CROWN GALL

A. Daisies in second immunity and susceptibility test. The 4th series of cuttings galled. These plants were inoculated with Resistant Daisy strain December 15, 1914. Photographed May 3, 1915. Typical plants from the lot of 27, the group photograph of which is shown in part in plate IA.

B. Daisy plants. Controls in second immunity and susceptibility test. Two out of 12 plants inoculated with Resistant Daisy strain December 15, 1914. Photo. May 3, 1915. Typical plants from the group of 12 photographed the same day (Pl. IB). These plants were from cuttings of plants which had never been galled.

SUMMARY

No permanent resistance to the crown gall organism was developed either in the Paris daisy or in rose bushes which were seedlings derived from somewhat resistant varieties. A temporary resistance or what at the time was supposed to be such was noted in the daisy cuttings in the middle of the first series, and in one plant of the three rose seedlings.

When the inoculations into Paris daisy cuttings made from galled plants were carried to the seventh generation, this resistance was lost and increased susceptibility to the disease was noted, but here a more virulent strain of the organism was used.

A second set of experiments with galled daisy cuttings seemed to indicate increased susceptibility to the disease in the fifth generation with lowered vitality of the whole plant.

Of the three rose seedlings produced from somewhat resistant plants one (Ophelia × Irish Fireflame) was very susceptible to crown gall, and one called the rose-red variety (Mrs. Charles Russell × Sunburst) appeared for a time quite resistant, that is for about a year. At the end of nearly two years this resistance had appreciably lessened although the variety never showed the same ability to form galls as did the other two seedling varieties.

THE ALTERNARIA BLIGHT OF POTATOES IN BERMUDA

H. H. Whetzel

With One Figure in the Text

The following observations on an epiphytotic of early blight of potatoes in Bermuda in the early winter of 1921, may be of some interest to plant pathologists.

The fall crop consisting chiefly of Bliss Triumph and a much smaller acreage of Garnet Chili is planted in Bermuda during the latter half of September and early October. In the autumn of 1921 weather conditions were very favorable to the growth of potatoes. Warm weather and abundant rainfall combined to produce a growth of crops which by early November gave promise of an extraordinary crop as indicated by the foliage which was profuse and of a dark green color.

Early in November the weather became increasingly wet, rain falling almost daily. About the middle of the month the early blight began to appear in many of the fields all through the colony on the earlier planted potatoes. It appeared first on the lower leaves on plants here and there throughout the fields, causing them to turn yellow and die. The lesions on these first infected leaves were quite characteristic for the disease as it commonly appears in the States. The lesions were rather small, definite in outline and with the usual target board markings. Then suddenly the tops in entire fields began to die off. This often began in spots or areas which rapidly enlarged, but usually the entire field was swept as by a fire.

From a distance these blighted fields had so much the appearance of a late blight attack that I felt sure it must be that disease. The suddenness of its development, the rapidity of its devastation and the "spots" in the field all argued for the Phytophthora blight. Moreover, on first entering one of these fields to make a careful examination I seemed confirmed in this opinion. The lesions (Fig. 1) were large, often an inch in diameter, the margins indefinite, dark and water-soaked. There were, to be sure, here and there the smaller definite target board lesions so characteristic of the Alternaria blight, but they were chiefly on the lower older leaves while the large indefinite lesions were abundant all over the foliage even to the uppermost young leaves. Moreover, the green stalks showed numerous large, dark, watery lesions apparently those of the late blight.

There were, however, two disturbing features in these field symptoms. Search as I might, not a trace was to be found of the white mildew zone so characteristic of the under surface of late blight lesions. This seemed remarkable for the ground was soaked and the foliage wet with the rains of the preceding night. Every condition requisite for an abundant production of the conidiophores of *Phytophthora infestans* seemed to obtain, but none was in evidence even under a hand lens. The

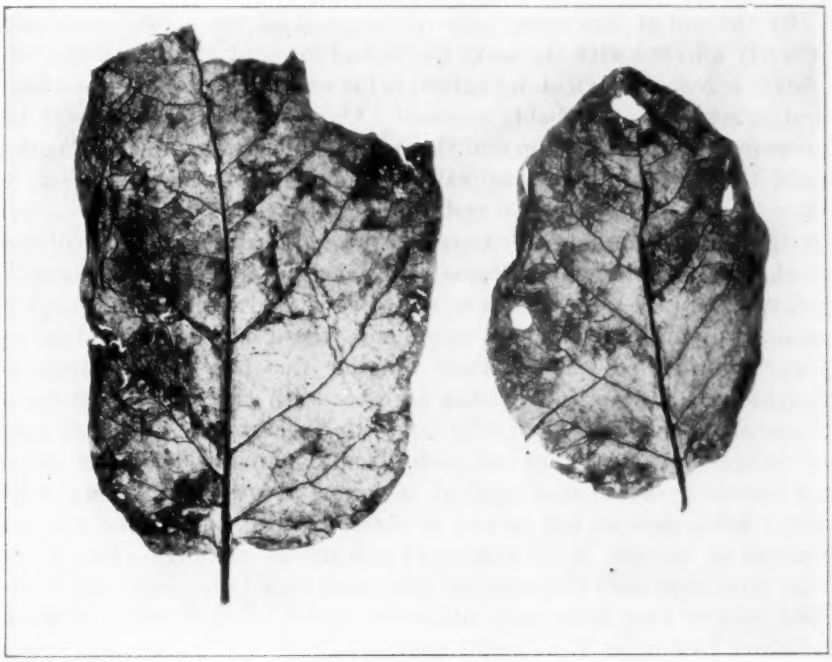


FIG. 1. Alternaria blight lesions on potato leaflets. Note indefinite water-soaked character of the lesions and absence of zonation. Contact photograph from dried specimens.

other puzzling thing was the slightly brownish hue and occasional traces of zonation in some of the large lesions. This together with the frequent presence of typical early blight lesions on the same leaflet caused me to hesitate over a final decision.

Mr. M. E. Luther, a recent graduate of the Wisconsin Agricultural College, had brought me to this particular field to diagnose the trouble and stood by awaiting my verdict. After audibly changing my mind two or three times, I finally said it must be the late blight, though the

symptoms certainly lacked some of the usual features of that disease. I took the precaution, however, to gather a quantity of the affected "trees"[1] which I at once took to the laboratory for microscopic examination. To my astonishment the lesions all showed an abundance of the spores of *Alternaria solani* and not a trace of the conidiophores or conidia of *P. infestans*.

Repeated microscopic examinations of blighted leaves from different fields always yielded like results.

By the end of November fully one-third of all the potato fields were severely affected with the early blight and in many the tops were completely killed. The yield in blighted fields was reduced at least one-third and in some cases probably one-half. Only a small percentage of the growers spray the fall crop and those who do delay the first application until the "trees" have practically completed their growth. That is, they do not begin until the early blight is often starting or even well along. A few who this year sprayed early and thoroughly held the disease in check to a considerable degree. However, as spraying was generally delayed, only the upper leaves were protected and as not more than two or three applications at most were made, the disease developed on the lower unprotected leaves. Poor spraying due largely to antiquated nozzles and low pressure pumps together with the washing effects of frequent rains combined to offer but little check to the disease in most of the sprayed fields. The best yields from blighted fields did not exceed six barrels to one of seed planted, including all grades of tubers, while many fields gave as low as two or three to one. In all cases the percentage of seconds, third grade and cull tubers was high. One of the best potato growers who sprayed early and very thoroughly and whose field showed very little early blight harvested seven to one. A severe wind on December 5, 6, and 7 destroyed the tops in this field which accounts for the relatively low yield, but it serves as a rough standard by which to judge of the effect in neighboring fields killed by the blight shortly before the wind storm.

This attack of early blight was not especially associated with maturity of the tops, tip burn or other injuries to the foliage. The foliage when the epidemic broke out was in perfect condition and in a vigorous state of growth. The weather had been ideal for growth of the "trees." It was a clear case of a virulent attack of an independent pathogene upon a normal, vigorous host.

[1] A colloquial but universal use of the term in Bermuda. Applied to the tops of such herbaceous plants as potatoes, lilies, celery and onions.

Those features of this epidemic which strike me as extraordinary in my experience with early blight are: (a) the suddenness and rapidity of its development, (b) the completeness of its devastation, (c) the size of the leaf lesions and their similarity to those of the late blight, and (d) the development of large water soaked lesions on the stalks.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY.

SEED TRANSMISSION OF LETTUCE MOSAIC

ALLAN G. NEWHALL

As one of the minor diseases of field-grown head lettuce in Western New York, mosaic has been observed for several years. It is commonly present to the extent of from 3 to 6 per cent, but in dry seasons, when insects are abundant, it is much more prevalent. Such was the case in 1921. From 10 to 15 per cent of the heads of the second crop in Wayne County became affected by September. The disease was also very prevalent in other lettuce growing sections of the State. Its spread was easily correlated with a general infestation of aphids, these insects having been found by Jagger (8), in 1919 to be capable of transmitting the virus.

During the summer of 1921, repeated search was made for wild hosts which might aid in the dissemination of the virus or be responsible for carrying it through the winter and furnishing the primary inoculum in the spring. A number of attempts were made by Dr. H. W. Dye and the author to transfer mosaic from milk-weed (*Asclepias syriaca*), and from water dock (*Rumex britannica*) to lettuce by means of rubbing, transferring bits of diseased tissue, and by hypodermic injections. Toward the end of the season cages were built and aphids employed, but no success attended any of these trials.

For several reasons it was suspected that lettuce mosaic was carried over in the seed. In the first place an occasional mosaic plant was found in the flats planted with commercial Big Boston or Romaine seed in the greenhouse. In the second place the disease was observed in the field in May and June soon after the young seedlings developed their third or fourth true leaf. At this time aphids are not prevalent, nor are they commonly found even later on such small lettuce. In the third place, seed transmission was demonstrated to occur in a number of mosaic diseases of vegetables and field crops. Reddick and Stewart (11), and Nelson (10), have reported seed transmission of the common bean mosaic, while Gardner and Kendrick (6) found the soy bean mosaic was transmitted in a similar manner. McClintock (9) has reported seed transmission of lima bean mosaic, while Doolittle (4) and Doolittle and Gilbert (5) found the cucumber mosaic could live over in the seed of the wild cucumber (*Micrampelis lobata*), and possibly in rare instances in that of the cultivated form. Dickson (2) and Dickson McRoxtie (3) report positive results of "seed inheritance"[1] in the mosaic of several

[1] There is no indication that the disease appears in the offspring in a definite ratio or any implication by them that it is necessarily carried in the embryo.

legumes, notably *Trifolium pratense*, *T. hybridum*, *Melilotus alba* and *Pisum sativum*, as well as Hippeastrum species. Miss Westerdijk (12) has furthermore reported a possible case of seed transmission of tomato mosaic, but the work of Allard (1), Dickson (2), and of Gardner and Kendrick (6) does not support her view.

In order to test the possibility of seed transmission of lettuce mosaic, twelve heads of Big Boston showing typical symptoms of the disease were carefully selected in the field at cutting time, staked, and allowed to go to seed. Seed was harvested from each of these individuals separately. To test its viability a small amount was sown immediately in the field and, out of 37 plants which grew, two showed distinct mosaic symptoms one month later. This was not considered important at the time, since no precautions were taken against possible insect inoculation.

GREENHOUSE TESTS

The following winter (1921–1922) seed from 3 of the 12 selected plants was sown in fresh loam, not known to have previously grown any lettuce. After 28 days, mosaic symptoms were distinctly apparent on 27 plants out of the total population of 563 plants which grew. Several doubtful cases were noted but not counted. As a few aphids and other insects were found in the vicinity of the lettuce bench, another test was made. This time every precaution was used to eliminate all other possible agents of transmission. The soil was steam sterilized, the seed bed was covered with cheesecloth after sowing, April 5, and the house fumigated several times during the next three weeks. On examination 24 days later, April 29, a considerable number of typical cases of mosaic had appeared. A diligent search was made for aphids and other insects but none were found. On May 30, the plants were pulled one at a time and the following counts made.

Parent's number	Population grown	No. of Mosaic plants
L—3	410	16
L—4	40	0
L—5	169	2
L—7	170	1
L—8	200	4
L—9	101	3
L—11	175	6
L—12	200	13
Totals	1465	45%

In addition 35 doubtful cases were held for three weeks more, since under greenhouse conditions mosaic symptoms may be more or less masked and slow in developing. Six of these developed more definite symptoms.

Since making these tests, additional field observations in Western New York have been made the past season. Several fields of lettuce in Wayne County were examined carefully in different places in July 22. The plants were not over 18 days old and were just getting their second or third true leaf. One hundred plants were counted in each of a number of different places in three fields, and in every case from one to three plants showed the first symptoms of mosaic. No insects were found on any of these plants as the season was very wet and the plants very small.

CONCLUSIONS

These tests and field observations seem to prove that mosaic of lettuce is frequently transmitted through the seed.

Probably the seed is the most important source of primary inoculum in the spring.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.

LITERATURE CITED

(1) ALLARD, H. A. The mosaic disease of tomatoes and petunias. Phytopath. **6:** 328–335, 2 fig. 1916.

(2) DICKSON, B. T. Studies concerning mosaic diseases. MacDonald Coll. Tech. Bull. 2. 125 p. 1922.

(3) ———— AND G. P. MCROSTIE. Further studies on mosaic. (Abstract) Phytopath. **12:** 42. 1922.

(4) DOOLITTLE, S. P. The relation of wild host plants to the overwintering of cucurbit mosaic. (Abstract) Phytopath. **11:** 47. 1921:

(5) ————AND W. W. GILBERT. Seed transmission of cucurbit mosaic by the wild cucunber. Phytopath. **9:** 326–327. 1919.

(6) GARDNER, M. W. AND J. B. KENDRICK. Soybean mosaic. Jour. Agric. Res. **22:** 111–114. Pl. 18–19. 1921.

(7) ———— ———— Overwintering of tomato mosaic. Bot. Gaz. **73:** 469–485. Pl. 17. 1922. Literature cited, p. 484–485.

(8) JAGGER, I. C. A transmissible mosaic disease of lettuce. Jour. Agric. Res. **20:** 737–740. Pl. 87. 1921.

(9) MCCLINTOCK, J. A. Lima bean mosaic. (Abstract) Phytopath. **7:** 60–61. 1917.

(10) NELSON, RAY. Transference of the bean mosaic virus by Macrosiphum solanifolii. Science **56:** 342–344. 1922.

(11) REDDICK, DONALD, AND V. B. STEWART. Varieties of beans susceptible to mosaic. Phytopath. **8:** 530–534. 1918.

(12) WESTERDIJK, JOHANNA. Die Mosaikkrankheit der Tomaten. Mededeel. Phytopath. Lab. "Willie Commelin Scholten," Amsterdam I. 20 p., 3 pl. (1 col. 1910.